A THESIS

entitled

PROSTAGLANDIN I_2 AND THE RENIN-ANGIOTENSIN SYSTEM

by

Lesley J. Brydon, B.Sc.

Submitted for the Degree of
Doctor of Philosophy
in
The University of Edinburgh

University of Edinburgh April 1984

To my husband Jim, and my parents Margaret and Norman

for their encouragement and moral support.

ABSTRACT

The work described in this thesis is concerned with the relationship between prostaglandin I_2 and the renin-angiotensin system.

In a conscious dog model, intravenous infusion of PGI_2 lowered systemic blood pressure but had very little effect on renal haemodynamics or renin release. It was postulated that in the dog, PGI_2 was not a potent renal vasodilator or renin-secretagogue.

Urinary 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, was quantified by gas chromatography-mass spectrometry. Using this method a linear relationship was demonstrated between circulating PGI_2 and urinary 6-keto-$PGF_{1\alpha}$ and a maximum rate of entry of PGI_2 into the circulation was estimated.

Following blockade of the renin-angiotensin system with the angiotensin I converting enzyme inhibitor captopril, systemic blood pressure fell and renal plasma flow and sodium excretion increased in both sodium replete and sodium deplete conscious dogs. These results emphasized the importance of the renin-angiotensin system in the control of systemic and renal haemodynamics under both sodium depleted and normal conditions. Urinary 6-keto-$PGF_{1\alpha}$ levels increased following captopril administration and showed a close correlation with the increase in renal plasma flow. It was suggested that PGI_2 may be involved in the renal vasodilator response to captopril but PGI_2 was not thought to participate in the vasodepressor, natriuretic or renin secretory actions of captopril.

Inhibition of angiotensin II formation by captopril had little effect on the hypotensive, renal vasodilator or renin secretory action of PGI_2 in either sodium replete or sodium deplete conscious dogs.

It was concluded that PGI_2 is not an important factor in the control of renin release in the conscious dog and that in comparison to angiotensin II, PGI_2 plays a very minor role in the control of systemic blood pressure and renal function.

The work detailed in this thesis was performed by myself (unless otherwise stated) in the Department of Pharmacology, University of Edinburgh, between 1979 and 1982.

ACKNOWLEDGEMENTS

I would like to thank Drs M.L. Watson and R.L. Jones for their supervision and encouragement.

I gratefully acknowledge the assistance of Mrs. B. Sanger for performing the plasma renin estimations and Mrs. P. Swan and Mr. L. Grant for their assistance with the flame photometry and auto-analyser techniques. Thanks are also due to Mr. C.G. Marr, Mr. P. Whelpdale and Miss A. McKay for their technical assistance.

I would also like to thank Mrs. J. Hunter and her staff in the animal house for their dedicated attention to the care of the animals used in this thesis.

Finally, I would like to thank Mrs. F. Anderson for her excellent typing of the manuscript.

The work described in this thesis was performed during the tenure of an Edinburgh University, Medical Faculty Scholarship to myself and a British Heart Foundation Research Grant to Drs. M.L. Watson and R.L. Jones.

CONTENTS

ABBREVIATIONS

sec	-	second
min	-	minute
hr	-	hour
kg	-	kilogram
mg	-	milligram
µg	-	microgram
ng	-	nanogram
l	-	litre
ml	-	millilitre
µl	-	microlitre
mM	-	milliMolar
µM	-	microMolar
$t_{\frac{1}{2}}$	-	half-life
SBP	-	systemic blood pressure
HR	-	heart rate
RPF	-	renal plasma flow
GFR	-	glomerular filtration rate
FF	-	filtration fraction
$U_{Na}V$	-	urinary sodium excretion
$U_{K}V$	-	urinary potassium excretion
$\dot{V}$	-	urine flow
$U_{osm}V$	-	urinary solute excretion
CH_2O	-	free water clearance
PRA	-	plasma renin activity
RIA	-	radioimmunoassay
GC-MS	-	gas chromatography - mass spectrometry

PGE_2 - prostaglandin E_2

PGI_2 - prostaglandin I_2

6-keto-$PGF_{1\alpha}$ - 6-keto-prostaglandin $F_{1\alpha}$

SECTION I

General Introduction

1.1 Prostaglandin Biosynthesis

The prostaglandins are a family of cyclopentane fatty acids. They have been found to occur naturally in almost every animal species and in every cell or tissue studied. Many naturally occurring prostaglandins and chemically synthesised analogues possess high biological potency and show a great diversity of pharmacological effects (for review, see Horton, 1979).

Prostaglandins are biosynthesised from the polyunsaturated fatty acids cis-8,11,14-eicosatrienoic (dihomo-γ-linolenic acid), cis-5,8,11,14-eicosatetraenoic acid (arachidonic acid) and cis-5,8,11,14,17-eicosapentaenoic acid which give rise to the mono-, bis- and trienoic prostaglandins respectively. Arachidonic acid is the most common fatty acid precursor of prostaglandins and is incorporated as a structural component of phospholipids into cell membranes and other sub-cellular structures of all tissues of the body. Arachidonic acid is released from cell membranes by the action of phospholipases, which can be activated by changes in the chemical environment. Once released from the membrane phospholipids, arachidonic acid is metabolised by two types of enzyme. One type of enzyme is a series of lipoxygenases which peroxidise arachidonic acid to form unstable hydroperoxides. These hydroperoxides break down to form the stable hydroxyacids or are further transformed into other products, e.g. leukotrienes. The other enzyme is a cyclo-oxygenase which oxidises arachidonic acid to form the unstable endoperoxides PGG_2 and PGH_2. These endoperoxides break down enzymatically or non-enzymatically to the stable substances PGA_2, PGB_2, PGC_2, PGD_2, PGE_2 and $PGF_{2\alpha}$ as well as 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and malionaldehyde (Figure 1.1). The biosynthetic pathways of PGA

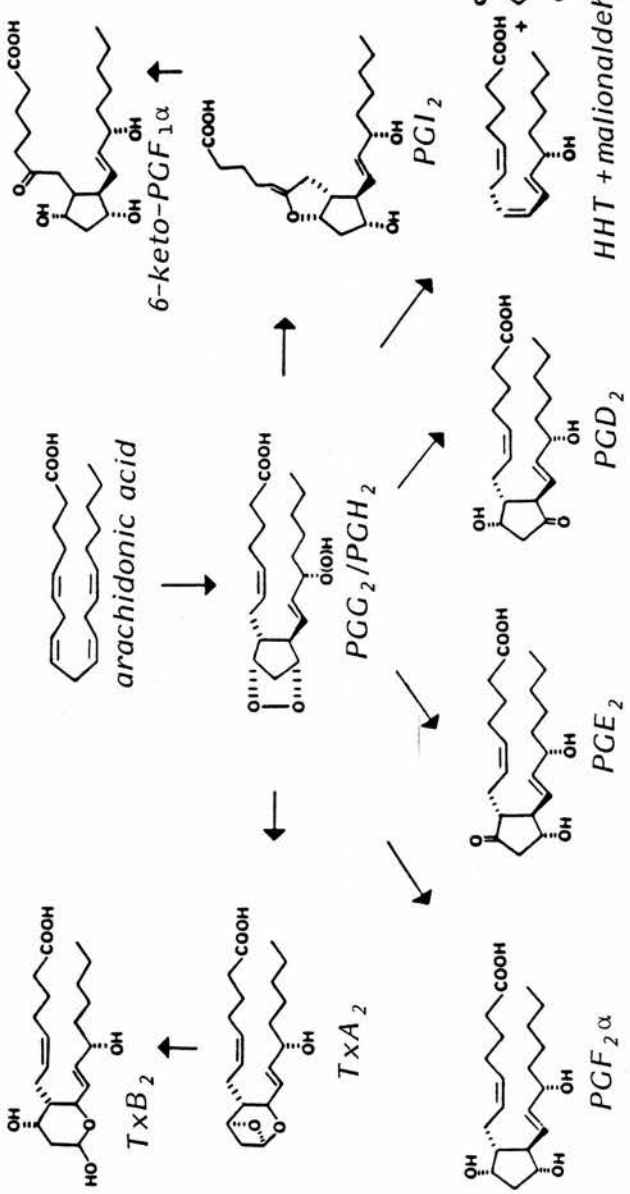


FIGURE 1.1: Principal pathways of prostaglandin/thromboxane biosynthesis.

and PGB compounds have not been elucidated but they are easily formed chemically from PGE compounds by action of base or acid. The prostaglandin endoperoxides are also enzymatically transformed into two other unstable products, thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) (Figure 1.1). Unlike PGE_2, PGD_2 and $PGF_{2\alpha}$, these products cannot result from chemical breakdown. TxA_2 ($t_{\frac{1}{2}}$ = 32 sec at 37°C) is the intermediate in the formation of the stable TxB_2 and is a potent aggregator of blood platelets. PGI_2, an unstable enol-ether ($t_{\frac{1}{2}}$ = 2-3 min at 37°C) is the intermediate in the formation of the stable 6-keto-$PGF_{1\alpha}$ which is in equilibrium with its lactol form (Figure 1.2). PGI_2 is the main metabolite of arachidonic acid in vascular tissue and is a potent inhibitor of platelet aggregation (for review see Moncada and Vane, 1978).

The prostaglandin content of most tissues and body fluids is in the range 10^{-10} to 10^{-8} gram per gram of tissue, although the capacity to synthesise and release prostaglandins far exceeds the basal levels detected (for review see Cuthbert, 1973). It can be concluded that prostaglandins are not stored and release can be equated with *de novo* biosynthesis. The release of essential fatty acids from the stores bound in phospholipids is probably the rate-limiting step in prostaglandin biosynthesis.

Prostaglandin biosynthesis can be inhibited by steroidal anti-inflammatory drugs, e.g. dexamethasone . These steroids inhibit the action of the phospholipase and prevent the release of arachidonic acid. Prostaglandin synthesis can also be inhibited by non-steroidal anti-inflammatory drugs, e.g. indomethacin and aspirin. These drugs inhibit the action of the cyclo-oxygenase enzyme and therefore prevent the conversion of arachidonic acid into its prostaglandin metabolites (for review see Flower, 1974).

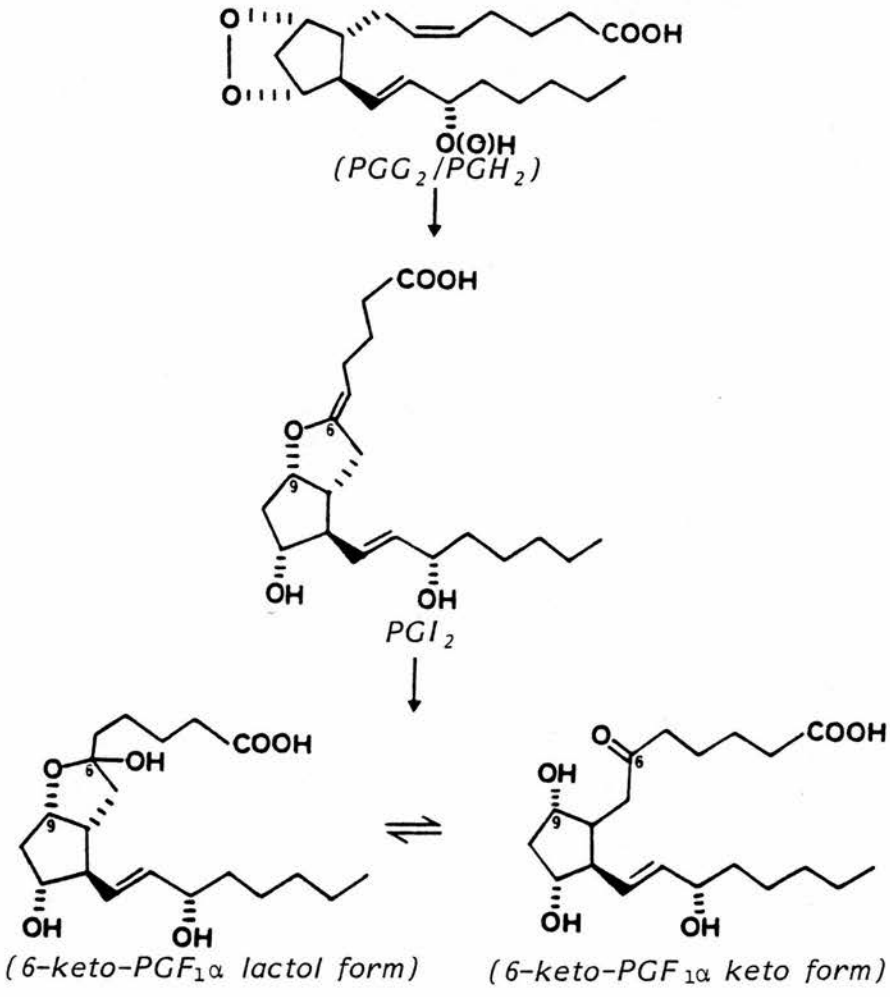


FIGURE 1.2: Transformation of endoperoxides into PGI_2 and 6-keto-$PGF_{1\alpha}$.

1.2 Renal Prostaglandin Production

In 1965, Lee *et al*. demonstrated the existence of several vaso-depressor acidic lipids in extracts of rabbit renal medulla (Lee, Covino and Takman). These acidic lipids were later characterised as PGE_2, $PGF_{2\alpha}$ and PGA_2 (Lee, Growshaw, Takman, Attrep and Gougoutas, 1967; Daniels, Hinman, Leach and Muirhead, 1967). The biosynthesis of PGE_2 and $PGF_{2\alpha}$ by renal medulla microsomes has been confirmed by many studies but enzymatic biosynthesis of PGA_2 could not be demonstrated in rabbit kidney microsomes (Blackwell, Flower and Vane, 1975) and GC-MS analysis did not detect PGA_2 in either the medulla or cortex of rabbit kidney (Larsson and Anggard, 1976). Since PGE_2 dehydrates rapidly to PGA_2, it is probable that the previously identified PGA_2 was formed artifactually from PGE_2 during the isolation procedure. PGD_2 has been detected following incubation of rabbit kidney microsomes with arachidonic acid (Blackwell *et al*., 1975).

Although it was originally thought that the renal cortex was unable to synthesise prostaglandins (Crowshaw and Szlyk, 1970), it was later demonstrated that rabbit cortical microsomes had a small capacity to synthesise PGE_2 (Anggard, Bohman, Griffin, Larrson and Maunsbach, 1972). Further dissection of the kidney revealed that prostaglandin synthesising capacity increased from the cortex towards the inner medulla (Larsson and Anggard, 1973). Within the medulla, histochemical and immunochemical localisation of prostaglandin synthetase demonstrated highest enzyme activity to be localised in the collecting tubules with less activity in the medullary interstitial cells (Janszen and Nugteren, 1971; Smith and Wilkin, 1977).

Zenser *et al*. identified 6-keto-$PGF_{1\alpha}$, reflecting PGI_2, in homogenates of rat renal cortex following incubation with PGH_2 but only negligible traces were found in the medulla (Zenser, Herman, Gorman and Davis, 1977). Rabbit cortical microsomes, incubated with either PGH_2 or arachidonic acid were found to synthesise PGI_2 whilst the medullary microsomes synthesised almost exclusively PGE_2 (Whorton, Smigel, Oates and Frolich, 1978). These studies led to attempts to compartmentalise renal prostaglandins into a PGI_2-vascular component and a PGE_2-tubular component. However, subsequent experimental evidence has not supported this theory. In rabbits the medulla has a greater capacity to synthesise a PGI_2-like substance than does the cortex (Silberbauer and Sinzinger, 1978). With a GC-MS technique, Oliw *et al*. found high levels of 6-keto-$PGF_{1\alpha}$ accumulated in cortex, medulla and papillae of post mortem rabbit kidney. Although the papillae 6-keto-$PGF_{1\alpha}$ level was highest, the cortex contained five times more 6-keto-$PGF_{1\alpha}$ than it did PGE_2 and $PGF_{2\alpha}$ (Oliw, Lunden, Sjoquist and Anggard, 1979). Homogenates of isolated rabbit collecting tubule cells produced predominantly 6-keto-$PGF_{1\alpha}$ unless incubated with arachidonic acid when PGE_2 was the major prostaglandin formed (Grenier and Smith, 1978). Contrary to this, cultured rabbit collecting tubule cells synthesised predominantly PGE_2 irrespective of incubating conditions (Grenier, Rollins & Smith, 1981; Schlondorff, Zanger, Satriano, Folkert and Eveloff, 1982). Sun *et al*. demonstrated that 6-keto-$PGF_{1\alpha}$ could only be detected when rabbit cortical and medullary microsomes were prepared under an atmosphere of argon to prevent inactivation of the PGI_2 synthetase by organic peroxides (Sun, Taylor, McGuire and Wong, 1981). Under these conditions, the medullary microsomes produced more PGI_2 than the cortical microsomes.

Within the cortex, immunohistochemical localisation of the prostaglandin synthetase has revealed the highest enzyme activity to be in the endothelial cells of the arteries and arterioles with lesser activity in the epithelial cells of the collecting tubules (Smith and Bell, 1978). The prostaglandin synthetase activity of the cortical collecting tubules was found to be less than in the medullary collecting tubules. Terragno *et al*. demonstrated that the arteries and arterioles isolated from pig kidney cortex by microdissection had a high capacity for PGI_2 biosynthesis (Terragno, McGiff and Terragno, 1978). Cultured rat glomerular epithelial and mesangial cells produce primarily PGE_2 (Petrulis, Aikawa and Dunn, 1981; Scharschmidt and Dunn, 1983). Incubation of these cells with arachidonic acid preferentially stimulates PGE_2 synthesis. Some investigators using post mortem rat isolated glomeruli have found PGE_2 to be the major prostaglandin produced (Sraer, Sraer, Chansel, Rusio-Marie, Kouznetzora and Ardoullou, 1979; Sraer, Siess, Moulonquet-Doleris, Oudinet, Dray and Ardoullou, 1982) whilst others found PGI_2 and $PGF_{2\alpha}$ to be the principal prostaglandins synthesised (Schlondorff, Roczniak, Satriano and Folkert, 1980; Kreisberg, 1982). Although TxA_2 was thought to be produced in the kidney only in conditions of renal damage (Morrison, Nishikawa and Needleman, 1977), several studies have identified TxB_2 in glomerular homogenates (Hassid *et al*., 1979; Sraer *et al*., 1982; Petrulis *et al*., 1981).

There have been few studies on renal prostaglandin production in the dog or human. Satoh and Satoh demonstrated that dog cortical and medullary microsomes synthesised predominantly PGI_2 but that the addition of glutathione to the incubation increased PGE_2 production at the expense of PGI_2 (Satoh and Satoh, 1980). A recent study investigating prostaglandin production by dog renal artery, vein, cortex and outer and inner

medulla demonstrated that in all zones, PGI_2 production was higher than that of PGE_2 or $PGF_2\alpha$. The vessels synthesised almost exclusively PGI_2 but the inner medulla had the highest capacity to synthesise PGI_2 (Okahara, Imanishi& Yamamoto, 1983). Human cortical and medullary microsomes incubated with arachidonic acid were found to synthesise PGI_2 and $PGF_2\alpha$ with very little PGE_2. The medulla showed a greater synthetic capacity than the cortex (Hassid and Dunn, 1980).

The literature has demonstrated qualitative and quantitative inconsistencies in prostaglandin production by different regions of the kidney and by different animal species. These inconsistencies most probably arise from differences in the *in vitro* experimental conditions. It seems likely that medulla and cortex contain isomerases for the production of PGE_2 and PGI_2. These two enzymes, however, have wide differences in substrate affinity and cofactor requirements. Therefore, any difference in substrate concentration, incubation time, or the presence of cofactors used in each study may lead to differences in prostaglandin product distribution. Furthermore, if the cyclooxygenase in an enriched tissue such as the kidney medulla, is allowed to operate at optimal conditions, the endoperoxide generated may be more than its isomerases can consume. The excess will randomly decompose to PGE_2, $PGF_2\alpha$ and PGD_2. This may explain why in several studies the production of PGE_2 and $PGF_2\alpha$ was apparently much higher than that of PGI_2. It is possible that *in vivo*, the endoperoxide metabolising enzymes are coupled with specific cyclo-oxygenases for efficient and rapid conversion of released arachidonic acid to specific products.

1.3 Metabolism and Urinary Excretion of Prostaglandin

PGE_2 and $PGF_{2\alpha}$ have a half-life of less than 30 seconds in the bloodstream due to their rapid degradation by the enzymes 15-hydroxy-prostaglandindehydrogenase and prostaglandin $^{13}\Delta$ reductase. Oxidation of the 15-OH group to the corresponding 15-keto group by 15-hydroxy dehydrogenase is the first step in prostaglandin degradation and, in general, the 15-keto metabolites have only $\frac{1}{10}$th the biological activity of the parent prostaglandin (Pike, Kupiecki and Weeks, 1967). Highest 15-hydroxyprostaglandin dehydrogenase activity has been demonstrated to be in the lung, renal cortex and spleen (Anggard, Larsson and Samuellsson, 1971; Larsson and Anggard, 1973) and more than 90% of intravenously infused PGE_2 or $PGF_{2\alpha}$ is removed in one circulation through the lungs (Ferreira and Vane, 1967). Highest prostaglandin $^{13}\Delta$ reductase activity is found in the adipose tissue, spleen, renal cortex and liver (Anggard *et al.*, 1971). In man, the main PGE_2 and $PGF_{2\alpha}$ metabolites in the blood are 15-keto-13,14-dihydro-PGE_2 and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (Hamberg and Samuellsson, 1971; Granström, 1972). Further steps in the metabolic pathway proceed at a comparatively slower rate. The 15-keto-13,14-dihydro metabolites are transformed by a series of β-oxidation and ω-oxidation reactions into a number of more polar derivatives which are excreted in the urine and faeces. The β-oxidation processes occur in the liver (Hamberg, 1968). kidney and lung (Nakano and Morsy, 1971). PGE and PGF metabolites, differing in the degrees of β-oxidation and ω-oxidation have been identified in the urine, and in man, the major urinary metabolites of PGE_2 and $PGF_{2\alpha}$ are 7α-hydroxy-5,11-diketo-tetranor-prostane-1,16-dioic acid and 5α,7α,dihydroxy-11-keto-tetranor-prostane-1,16-dioic acid, respectively (Hamberg and Samuelsson, 1971; Granström and Samuelsson, 1971).

PGE_2 and $PGF_{2}\alpha$ can also be interconverted enzymatically within the kidney. A 9-keto-reductase has been isolated in rabbit renal cortex which reduces PGE_2 to $PGF_{2}\alpha$ (Stone and Hart, 1975). The activity of this enzyme is thought to be positively correlated to sodium intake (Weber, Larsson and Scherer, 1977). A 9-hydroxy prostaglandin dehydrogenase has also been isolated from mammalian renal cortex (Pace-Asciak, 1975; Moore and Hoult, 1978). This enzyme oxidises 15-keto-13,14-dihydro$PGF_{2}\alpha$ to 15-keto-13,14-dihydroPGE_2 in most species but has only been shown to oxidise $PGF_{2}\alpha$ directly to PGE_2 in rabbit kidney (Hoult and Moore, 1977). The physiological significance of these inter-conversions between PGE_2 and $PGF_{2}\alpha$ remains unclear.

The high capacity of the lung and kidney to metabolise prostaglandins leaves little opportunity for PGE_2 or $PGF_{2}\alpha$ in the circulation to enter the urine in an unmetabolised form. Intact PGE_2 and $PGF_{2}\alpha$ in the urine has been demonstrated to be of renal origin (Frolich, Wilson, Sweetman, Smigel, Nies, Carr, Watson and Oates, 1975) and measurement of urinary PGE_2 and $PGF_{2}\alpha$ levels taken to reflect solely renal synthesis of the prostaglandins. Infusion of large quantities of PGE_2 or $PGF_{2}\alpha$ into the renal artery results in the appearance of these prostaglandins in the urine. They enter the urine primarily by secretion via the organic acid pathway in the proximal tubule and also by filtration at the glomerulus (Rennick, 1977; Rosenblatt, Patak and Lifschitz, 1978). However, substantial amounts of the prostaglandins infused into the kidney are metabolised prior to excretion presumably due to ready access to cortical structures through the glomerular and peritubular capillaries. Using a stop-flow technique, Williams *et al*. demonstrated that endogenous PGE_2 and $PGF_{2}\alpha$ enters the nephron distal to the proximal tubule and suggests that the loop of Henlé is the major site of entry of PGE_2 and

$PGF_{2\alpha}$ into the urine (Williams, Frolich, Nies and Oates, 1977). It seems likely, therefore, that PGE_2 and $PGF_{2\alpha}$ synthesised in the renal medulla enter the nephron at the loop of Henlé and are excreted in the urine unmetabolised. PGE_2 and $PGF_{2\alpha}$ reaching the kidney via the renal artery are mostly metabolised in the renal cortex. However, they may enter the urinary space by filtration at the glomerulus and secretion in the proximal tubule.

PGI_2 is a good *in vitro* substrate for 15-hydroxyprostaglandin-dehydrogenase (McGuire and Sun, 1978). However, *in vivo*, 35-65% of an infusion of PGI_2 escapes inactivation in one complete circulation (Dusting, Moncada and Vane, 1978). Infusion of PGI_2 through the liver or hindlimb results in partial inactivation similar to that demonstrated with PGE_2. However, infusion of PGI_2 through the lung does not result in inactivation. It has therefore been proposed that PGI_2 is a poor substrate for the lung uptake mechanism. It was originally thought that 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, was not a substrate for 15-hydroxyprostaglandindehydrogenase (McGuire and Sun, 1978). However, the presence of monoketo and diketo metabolites in rat urine following both PGI_2 and 6-keto-$PGF_{1\alpha}$ infusion, suggests that although 6-keto-$PGF_{1\alpha}$ is a poor substrate, it does undergo 15-dehydrogenation (Sun and Taylor, 1978; Sun, Taylor, Sutter and Weeks, 1979).

PGI_2 is metabolised by the blood vessels (Wong, Sun and McGiff, 1978), kidney (Wong, McGiff, Cagen, Malik and Sun, 1979) and liver (Wong, Malik, Desiderius, McGiff and Sun, 1980). The blood vessels are very rich in 15-hydroxyprostaglandindehydrogenase and the main metabolic product of PGI_2 is 6,15-diketo-$PGF_{1\alpha}$. In the kidney, there are two metabolic pathways: (1) PGI_2 is hydrolysed to 6-keto-$PGF_{1\alpha}$ which is then β-oxidised to 2,3-dinor-6-keto-$PGF_{1\alpha}$ and (2) PGI_2 and/or

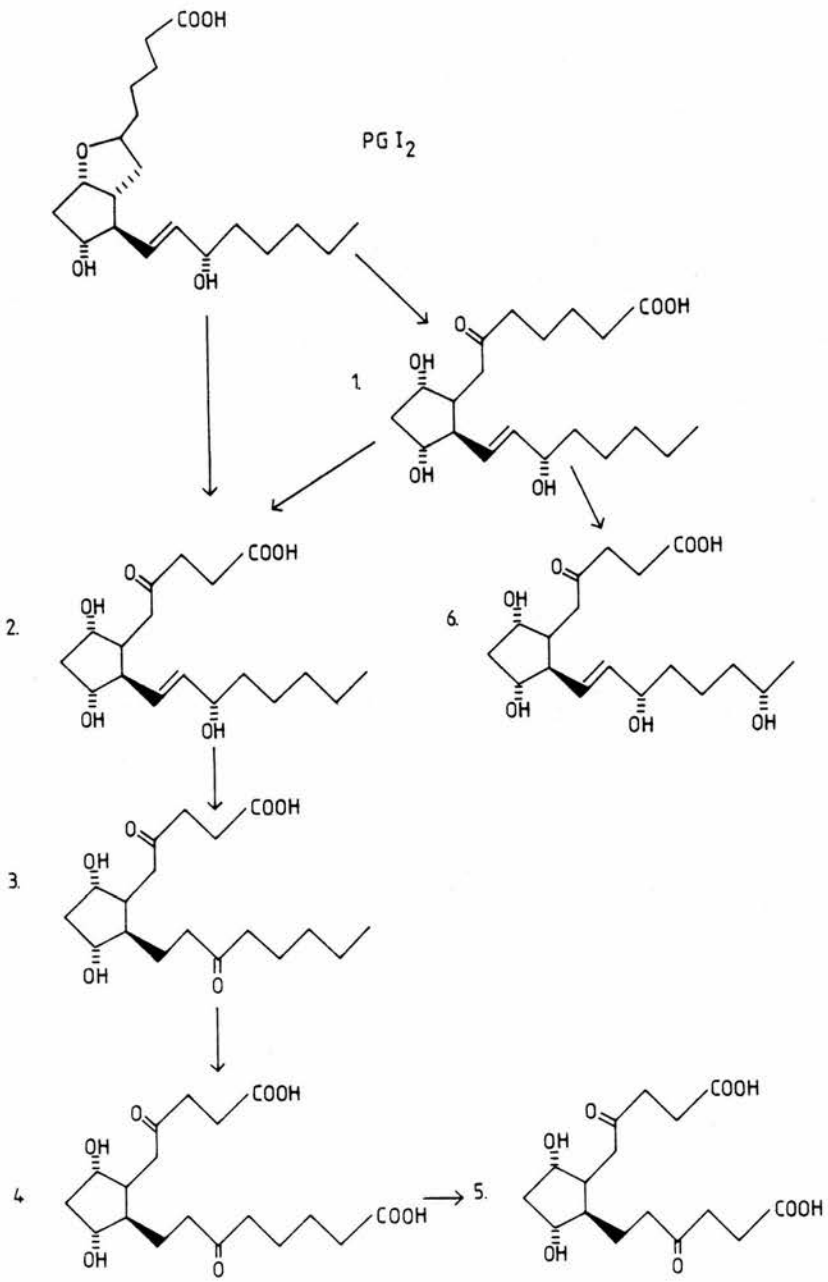


FIGURE 1.3: Main urinary metabolites of PGI_2.

1. 6-keto-$PGF_{1\alpha}$.
2. 2,3-dinor-6-keto-$PGF_{1\alpha}$.
3. 2,3-dinor-6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$.
4. 2,3-dinor-6,15-diketo-13,14-dihydro-20-carboxyl-$PGF_{1\alpha}$.
5. 2,3,19,20-tetranor-6,15-diketo-13,14-dihydro-18-carboxyl-$PGF_{1\alpha}$.
6. 2,3-dinor-19-hydroxy-6-keto-$PGF_{1\alpha}$.

6-keto-$PGF_{1\alpha}$ are metabolised by 15-hydroxyprostaglandindehydrogenase and prostaglandin $^{13}\Delta$ reductase to 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ which is then β-oxidised to 2,3-dinor-6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$. In the liver, there is very little 15-hydroxyprostaglandindehydrogenase and metabolism takes place primarily by β oxidation and ω hydroxylation and oxidation. A 9-hydroxy prostaglandin dehydrogenase has been isolated in rabbit liver and human platelets which converts PGI_2 and/or 6-keto-$PGF_{1\alpha}$ to 6-keto-PGE_1 (Wong, Lee, Reiss and McGiff, 1980; Grans and Wong, 1981). This enzyme is not the same one that converts $PGF_{2\alpha}$ to PGE_2 in the rabbit renal cortex. 6-keto-PGE_1 has similar biological activity to PGI_2 (Quilley, Wong and McGiff, 1979).

Urinary metabolic profiles of PGI_2 have been determined in the rat (Sun and Taylor, 1978), monkey (Sun and Taylor, 1981), and in man (Rosenkranz, Fischer, Weimer and Frolich, 1980). Up to twelve different metabolites of PGI_2 have been identified in the urine and although the same metabolites have been identified in all three species the relative proportions of the different metabolites varies. The main urinary metabolites of PGI_2 are shown in Figure 1.3. In the rat, the primary metabolic pathway appeared to be via 15-dehydrogenation followed by substantial β and ω oxidation and the main urinary metabolite was 2,3,19,20-tetranor-6,15-diketo-13,14-dihydro-18-carboxyl-$PGF_{1\alpha}$. No unchanged 6-keto-$PGF_{1\alpha}$ was detected but 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 2,3-dinor-19-hydroxy-6-keto-$PGF_{1\alpha}$ also represented major urinary metabolites indicating that some PGI_2 was hydrolysed to 6-keto-$PGF_{1\alpha}$ without prior 15-hydrogenation. In the monkey and in man, 15-dehydrogenation appears to play only a small role in the metabolism of PGI_2, the major metabolic pathway being hydrolysis to 6-keto-$PGF_{1\alpha}$ followed by β-oxidation and 19 or 20 hydroxylation and oxidation to the dicarboxylic

acid. In the monkey, unchanged 6-keto-$PGF_{1\alpha}$ represents the main urinary metabolite of PGI_2 whilst in the human, 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 2,3-dinor-6,15-diketo-13,14-dihydro-20-carboxyl-$PGF_{1\alpha}$ are the main metabolites of PGI_2 in the urine.

Circulating 6-keto-$PGF_{1\alpha}$ can be excreted unchanged into the urine (Sun *et al.*, 1979; Rosenkranz et al. 1980a) and appears to be freely filtered at the glomerulus (Rosenkranz, Kitajima and Frolich, 1981a). Pretreatment with probenecid has no effect on the urinary excretion of 6-keto-$PGF_{1\alpha}$ suggesting that, unlike PGE_2 and $PGF_{2\alpha}$, it is not secreted into the tubule via an organic acid pathway (Rosenkranz *et al.*, 1981). However, no studies on other tubular secretory pathways have been performed and it is possible that in addition to glomerular filtration 6-keto-$PGF_{1\alpha}$ enters the urine at a site distal to the proximal tubule.

1.4 Prostaglandins and Renal Function

Infusion of PGE_1 and PGE_2 into the renal artery of anaesthetised dogs results in dose-dependent increases in renal blood flow, sodium excretion, urine volume and free water clearance. There is no change in glomerular filtration rate and variable change in potassium excretion (Vander, 1968; Stranhoy, Ott, Schneider, Willis, Beck, Davis and Knox, 1974). PGD_2 also increases renal blood flow when infused into the renal artery of anaesthetised dogs but has no consistent natriuretic or diuretic action (Fresinger , 1977; Bolger, Eisner, Shea, Ramwell and Slotkoff, 1977). In contrast, intrarenal infusion of $PGF_{2\alpha}$ has no consistent effect on renal blood flow or sodium and water excretion (Gross and Bartter, 1973). Infusion of arachidonic acid into the renal artery results in a detectable increase in prostaglandin levels in renal venous blood and increases renal blood flow, sodium excretion and urine

volume (Tannenbaum, Splawinski, Oates and Nies, 1975). Similar changes in renal function can be obtained following infusion of the prostaglandin endoperoxides PGH_2 and PGG_2 into the renal artery (Oliver, Sciacca and Cannon, 1979). Arachidonic acid and prostaglandin endoperoxides are, however, much less potent than exogenous PGE_2 in increasing renal blood flow. The reasons for these differences are most likely related to differences in access to sites of action. PGE_2 infused into the renal artery has direct access to the pre- and postglomerular resistance vessels and peritubular capillaries of all nephrons. However, PGE_2 is synthesised from arachidonic acid primarily in the renal medulla and is therefore only likely to affect the vessels of the juxtamedullary nephrons.

A reduction in p-aminohippurate extraction during intrarenal PGE_2 infusion suggested a redistribution of intrarenal blood flow (Vander, 1968; Gross and Bartter, 1973) and radioactive microsphere techniques have demonstrated that intrarenal infusion of PGE_2 or arachidonic acid causes a redistribution of blood flow towards the juxtamedullary cortex (Chang, Splawinski, Oates and Nies, 1975; Larsson and Anggard, 1974). Whilst such changes in renal haemodynamics could result in increased sodium excretion, there is evidence to suggest that PGE_2 may exert a direct action on the renal tubule to inhibit sodium reabsorption. Infusion of low concentrations of PGE_2 which do not increase renal plasma flow, still induce a natriuresis (Vander, 1968). Using *in vivo* micropuncture techniques, PGE_2 was demonstrated to have no effect on sodium transport in the rat proximal tubule or loop of Henlé but to decrease fractional sodium reabsorption along the distal nephron (Fulgraff and Meiforth, 1971). Using *in vitro* microperfusion techniques, PGE_2 was found to inhibit sodium transport in medullary and cortical collecting tubules of both normal and DOCA-salt loaded rabbits (Stokes and Kokko,

1977; Iino and Imai, 1978). PGE_2 was also found to inhibit chloride transport in the medullary thick ascending limb of Henlé but not in the cortical thick ascending limb (Stokes, 1979). Contrary to this, Fine and Trizna were unable to demonstrate any effect of PGE_2 on rabbit isolated medullary collecting tubules or medullary thick ascending limbs of Henlé (Fine and Trizna, 1977). Part of the discrepancy between these studies may be due to differences in experimental techniques and/or due to the heterogenecity of nephron segments. The question as to whether PGE_2 has a direct effect on the renal tubule to inhibit sodium reabsorption, remains unresolved.

The diuresis during intrarenal PGE_2 infusion is associated with an increase in free water clearance and decrease in fractional water reabsorption (Gross and Bartter, 1973; Tannenbaum *et al.*, 1975). This may be related to changes in medullary blood flow resulting in decreased proximal sodium reabsorption, increased delivery of sodium to the distal tubule and increased sodium reabsorption in the distal tubule with increased free water clearance. Alternatively, water excretion may be increased by direct antagonism of vasopressin-mediated water transport. Vasopressin increases water permeability by increasing cyclic AMP formation and accumulation within the epithelial cell. PGE_1 has been shown to inhibit the water permeability response to vasopressin but not to exogenous cyclic AMP in isolated rabbit collecting ducts (Grantham and Orloff, 1968). PGE_1 also reduces the accumulation of cyclic AMP in renal medulla slices exposed to vasopressin (Beck, Kaneto, Zeor, Field and Davis, 1971). Studies demonstrating that inhibition of prostaglandin synthesis with indomethacin enhanced the water permeability and medullary cAMP response to low concentrations of vasopressin (Anderson, Berl, McDonald andSchrier, 1975; Lum, Aisenbrey,

Dunn, Berl, Schrier and McDonald, 1977) together with the demonstration that PGE_2 was the major prostaglandin synthesised by the renal medulla (Larrson and Anggard, 1973) suggested that PGE_2 may be important in antagonising the actions of vasopressin. However, PGE_2 failed to inhibit an increase in cAMP in a rat medullary collecting tubule cell preparation incubated with vasopressin (Jackson, Edwards and Dousa, 1980). This lack of effect of PGE_2 may have been due to a biphasic action of PGE_2 on adenyl cyclase since it has been demonstrated that low concentrations of PGE_2 inhibit while high stimulate adenyl cyclase activity (Kather and Simon, 1979). However, PGE_2, over a range of concentrations, stimulated basal water permeability in isolated rat papillary collecting ducts (Ray and Morgan, 1981). The ability of PGE_2 to stimulate water permeability was much less than that of vasopressin and their effects were found not to be additive. It was therefore postulated that PGE_2 may act as a competitive partial agonist for vasopressin with weaker intrinsic effect. The evidence towards an action of PGE_2 to antagonise the effects of vasopressin remains unconvincing.

Infusion of PGI_2 into the renal artery of anaesthetised dogs results in an increase in total renal blood flow and a redistribution of blood flow away from the outer cortex to the juxtamedullary area. There is no change in glomerular filtration rate, therefore filtration fraction decreases. Intrarenal infusion of PGI_2 also increases sodium and potassium excretion but, unlike PGE_2, has no effect on free water clearance (Bolger, Eisner, Ramwell and Slotkoff, 1978; Gerber, Nies, Friesinger, Gerkens, Branch and Oates, 1978b; Hill and Moncada, 1979). Although Bolger *et al*. found PGI_2 and PGE_2 to be equipotent in causing renal vasodilatation, a comparison of the dose-response relationships of PGI_2 and PGE_2 infused into the renal artery of salt-loaded and salt-depleted

anaesthetised dogs revealed that PGE_2 was up to three times more potent than PGI_2 in increasing renal blood flow (Jones, Watson and Ungar, 1981). PGE_2 was also more potent than PGI_2 in increasing urine flow, sodium and potassium excretion and free water clearance. It is interesting to note that arachidonic acid infused into the renal artery is also less potent than PGE_2 in causing vasodilatation and natriuresis (Tannenbaum *et al.*, 1975). It may be that although PGE_2 is the more active prostaglandin, arachidonic acid in the kidney is converted preferentially to PGI_2.

There is some evidence to suggest that the natriuresis caused by intrarenal PGI_2 infusion is due to a direct effect of PGI_2 on the nephron. An increase in solute delivery to the distal tubule and a decrease in distal tubular sodium reabsorption has been demonstrated during intrarenal PGI_2 infusion in anaesthetised dogs (Gullner, Nicolaou and Bartter, 1980). However, these changes in sodium reabsorption may also be the consequence of alterations in renal haemodynamics during the PGI_2 infusion. An *in vitro* study demonstrated that PGI_2 could decrease sodium reabsorption in isolated rabbit cortical collecting ducts (Iino and Brenner, 1981). It seems likely, however, that the natriuresis is due, at least in part, to the increase in total renal blood flow and redistribution of intrarenal blood flow observed during PGI_2 infusion. Since, unlike PGE_2, PGI_2 did not increase free water clearance, it is unlikely that PGI_2 antagonises the actions of vasopressin on the collecting duct.

A change in the loop of Henlé flow rate of a single nephron induces an inverse change in glomerular filtration rate, a phenomenon generally referred to as tubuloglomerular feedback. This feedback response is blunted during inhibition of prostaglandin synthesis by indomethacin

(Schnermann, Schubert, Hermle, Herbst, Stowe, Yarimizu and Weber, 1979). Both PGI_2 and PGE_2 are capable of reversing the inhibition of the feedback response (Schnermann and Weber, 1982) but since PGI_2 is ten times more potent in its effect and is the major arachidonate metabolite in the cortex, it may be the main prostaglandin involved in the tubuloglomerular feedback mechanism. Other investigators, infusing PGI_2 without prior indomethacin failed to demonstrate an effect on tubuloglomerular feedback and at high concentrations of PGI_2, an attenuation of the feedback response was demonstrated (Boberg, Hahne and Persson, 1981). However, this attenuation was associated with a fall in systemic blood pressure and was thought to be a consequence of a PGI_2-induced change in vascular tone rather than a specific influence on the feedback pathway.

6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, has no effect on renal blood flow (Hill and Moncada, 1979). However, the stable PGI_2 metabolite, 6-keto-PGE_1, has been found to be equipotent with PGI_2 in increasing renal blood flow when infused into the renal artery of anaesthetised rats and dogs (Quilley *et al.*, 1979; Jackson, Herzer, Zimmerman, Branch, Oates and Gerkens, 1981). This has led to the proposal that 6-keto-PGE_1 may be responsible for some of the biological actions of infused PGI_2. However, an intravenous infusion of PGI_2 into human subjects which caused systemic haemodynamic changes, did not alter plasma levels of 6-keto-PGE_1 (Jackson, Goodman, Fitzgerald, Oates and Branch, 1981). It is therefore unlikely that 6-keto-PGE_1 is responsible for the renal vasodilatation observed during PGI_2 infusion.

Renal prostaglandin synthesis increases during conditions such as renal ischaemia (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee and Ng, 1970) and repeated renal nerve stimulation

(Dunham and Zimmerman, 1970) and in response to vasoactive peptides such as bradykinin (McGiff, Terragno, Malik, Lonigro, 1973) and angiotensin II (McGiff, Crowshaw, Terragno and Lonigro, 1970a). Under these conditions, prostaglandins are thought to help maintain renal blood flow. Evidence for a role of basal prostaglandin production in the control of renal haemodynamics and electrolyte excretion under normal conditions is conflicting.

Inhibition of prostaglandin synthesis by indomethacin or meclofenamate in the anaesthetised laparotamised dog results in a reduction in renal blood flow (Lonigro, Itskovitz, Crowshaw and McGiff, 1973). However, in the conscious dog at rest, indomethacin or meclofenamate administration has no effect on renal blood flow (Terragno, Terragno and McGiff, 1977; Zimmerman, 1978). When the renin-angiotensin system is stimulated by sodium depletion, inhibition of prostaglandin synthesis by indomethacin or meclofenamate in the conscious dog results in a reduction in renal blood flow (Blasingham, Shade, Share and Nasjletti, 1980; De Forrest, Davis, Freeman, Seymour, Rave, Williams and Davis, 1980). This suggests that prostaglandins only contribute to the control of renal blood flow during activation of the renin-angiotensin system. Investigations into the effect of prostaglandin synthesis inhibition on sodium excretion have produced similarly conflicting results. Administration of indomethacin or meclofenamate to conscious sodium replete dogs has been reported to increase, decrease or have no effect on sodium excretion (Kirschenbaum and Stein, 1976; Fejes-Toth, Mahyar and Walker, 1977; Zambraski and Dunn, 1979). The effect of prostaglandin synthesis inhibition on sodium excretion is not altered by sodium status since sodium excretion was unchanged following indomethacin or meclofenamate administration in both sodium replete and deplete conscious dogs (Blasingham *et al.*, 1980a; De Forrest *et al.*, 1980).

It would seem, therefore, that although exogenous infusion of prostaglandins, namely PGE_2 and PGI_2, significantly influences renal blood flow and sodium excretion, the physiological importance of the endogenous production of these prostaglandins remains unclear.

1.5 Prostaglandins and the Renin-Angiotensin System

1.5.1 Prostaglandins and renin release

The demonstration by Strong *et al.* that the renin and prostaglandin content were elevated in the ischaemic half of a kidney from a woman with renovascular hypertension suggested that prostaglandins may participate in the control of renin release (Strong, Boucher, Nowaczynski and Genest, 1966). Although Vander failed to demonstrate any effect of an intrarenal infusion of PGE_1 or PGE_2 on plasma renin activity in the anaesthetised dog (Vander, 1968), Werning *et al.* using a similar preparation observed an increase in plasma renin activity following infusion of PGE_1 into the renal artery (Werning, Vetter, Weidmann, Schweikert, Stiel and Siegenthaler, 1971). Infusion of arachidonic acid into the renal artery or the lower aorta of the anaesthetised rabbit, rat or dog results in an increase in plasma renin activity which can be prevented by prior administration of indomethacin (Larsson, Weber and Anggard, 1974; Weber, Holzgreve, Stephan and Herbst, 1975; Bolger, Eisner, Ramwell and Slotkoff, 1976). Several studies have demonstrated that PGE_2 infused into the renal artery of anaesthetised dogs results in an increase in plasma renin activity (Yun, Kelly, Bartter and Smith Jr., 1977; Osborn, Noordeiner, Hook and Bailie, 1978). Bolger *et al.* demonstrated that PGD_2 was equipotent with PGE_2 in increasing plasma renin activity when infused intrarenally in the

anaesthetised dog (Bolger *et al.*, 1977). Gerber *et al.* failed to observe any effect of PGD_2 on plasma renin activity but showed that PGI_2 infused into the renal artery of anaesthetised dogs was twice as active as PGE_2 in increasing renin release (Gerber, Branch, Nies, Gerkens, Shand, Hollifield and Oates, 1978a). The action of intrarenal and intravenous infusion of PGI_2 to increase plasma renin activity has been confirmed in both animals and man (Jones *et al.*, 1981; Patrono, Pugliese, Ciabattoni, Maseri, Chierchia, Peskar, Cinotti, Simonetti and Pierucci, 1982). A role for prostaglandins in the control of renin release has also been implied from studies demonstrating that indomethacin administration decreases basal plasma renin activity in man (Rumpf, Frenzel, Lowitz and Scheler, 1975; Frolich, Hollifield, Dormais, Frolich, Seyberth, Michelakis and Oates, 1976), conscious rabbits (Romero, Dunlop and Strong, 1976), rats (Suzuki, Franco-Saenz, Tan and Mulrow, 1981a) and in anaesthetised dogs (Seymour and Zehr, 1979). However, no effect of indomethacin could be demonstrated in conscious dogs (Blasingham *et al.*, 1980b).

Since infusion of arachidonic acid or prostaglandins into the renal artery also results in changes in renal haemodynamics and electrolyte excretion, isolated renal preparations have been used to study the direct effect of prostaglandins on renin release. Incubation of low concentrations of PGE_2 with a rabbit cortical cell preparation resulted in an increase in renin release although higher concentrations of PGE_2 had no effect. Incubation with $PGF_2\alpha$ resulted in an inhibition of renin release (Dew and Michelakis, 1974). Similarly, $PGF_2\alpha$ caused a dose-dependant inhibition of renin release when incubated with rabbit renal cortical slices (Weber, Larsson, Anggard, Hamberg, Corey, Nicolaou and Samuelsson, 1976). PGE_2 failed to have any effect on renin release

in this preparation although incubation of the cortical slices with either arachidonic acid or the endoperoxide PGG_2 resulted in an increase in renin release. Whorton *et al*. also failed to demonstrate any effect on renin release during incubation of rabbit renal cortical slices with PGE_2 but showed that incubation with PGI_2 resulted in a large increase in renin release (Whorton, Misono, Hollifield, Frolich, Inaganii and Oates, 1977). Later studies, however, demonstrated PGE_2 to be equipotent with PGI_2 in increasing renin release in rabbit and rat renal cortical slices and $PGF_{2\alpha}$ and PGD_2 to be inactive or weak agonists at high concentrations (Whorton, Lazar, Smigel and Oates, 1981; Suzuki *et al*., 1981b). 6-keto-$PGF_{1\alpha}$ has no effect on renin release either *in vitro* (Whorton *et al*., 1977) or *in vivo* (Jackson *et al*., 1980) but the enzymatically produced PGI_2 metabolite, 6-keto-PGE_1 has been demonstrated to be more potent than PGI_2 in increasing renin release when infused into the renal artery of anaesthetised dogs (Jackson *et al*., 1980), incubated with rabbit renal cortical slices (McGiff, Spokas and Wong, 1982) or infused into the isolated rat or rabbit kidney (Schwertschlag, Stahl and Hackenthal, 1982). The degree to which PGI_2 is metabolised to 6-keto-PGE_1 in the kidney *in vivo* is not known and therefore the physiological significance of the renin secretory activity of 6-keto-PGE_1 remains undefined. Since PGI_2 is the predominant prostaglandin synthesised in the renal cortex and has been shown to consistently increase renin release in both *in vivo* and *in vitro* preparations, it seems probable that it is this arachidonate metabolite which is most closely involved in the control of renin release.

Results from the isolated renal tissue studies demonstrated that prostaglandins could increase renin release by a direct effect on the juxtaglomerular cells. Other studies were undertaken to determine which

of the known intrinsic mechanisms of renin release were mediated by prostaglandins.

There are essentially three intrinsic mechanisms for renin release from the mammalian kidney (for review see Davis and Freeman, 1976). The renal vascular baroreceptor located in the afferent arteriole senses changes in either renal perfusion pressure or vascular wall tension to alter renin release such that a decrease in perfusion pressure increases renin release. The macula densa, located at the distal end of the cortical thick ascending limb of the loop of Henlé modulates renin release in response to alterations in the distal delivery of sodium chloride. There was originally controversy as to whether renin release was directly or inversely related to sodium transport at the macula densa. It is now accepted that a decrease in the distal tubular delivery of sodium chloride results in renin release, whereas an increase in distal tubular delivery of sodium chloride results in an inhibition of renin release. The afferent and efferent arterioles and the juxtaglomerular cells of the kidney are innervated by sympathetic fibres. The sympathetic nervous system participates in the release of renin such that stimulation of renal sympathetic nerves results in an increase in renin release. Renal nerve activity is controlled by several neural arc reflexes and accelerated renal sympathetic nerve activity has been implicated as a cause for the increased renin release that accompanies upright posture, exercise and psychosocial stimuli. The increase in renin release following renal sympathetic nerve stimulation is thought to be due primarily to the action of noradrenaline on β-adrenergic receptors. The role of α-adrenergic receptors in the control of renin release in response to renal sympathetic nerve stimulation is controversial. Some studies have demonstrated an increase in renin release in response to α-adrenergic activation

(Hong, 1980) and an inhibition by phentolamine of the increase in plasma renin activity after renal nerve stimulation (Cootes, Johns, MacLeod and Singer, 1972). Other investigators have failed to observe any effect of α-adrenergic receptor antagonists on the sympathetically mediated increase in renin release (Ganong, 1973) and *in vitro* studies have suggested that renal α-adrenergic stimulation may inhibit renin release (Vandongen and Peart, 1974).

Data *et al*. used an anaesthetised dog model to demonstrate a role for prostaglandins in the renal baroreceptor control of renin release (Data, Gerber, Crump, Frolich, Hollifield and Nies, 1978). In this model the renal baroreceptor is isolated from the macula densa and sympathetic mechanisms of renin release. Ligation of the ureter and occlusion of the renal artery of one kidney for two hours, two days before the study renders that kidney non-filtering, the contralateral kidney being excised on the day of the study. In the non-filtering kidney, the macula densa is not functional due to the cessation of tubular fluid flow. The effects of the sympathetic system and circulating catecholamines on renin release were abolished by renal denervation and a continuous infusion of propranolol. Under these conditions, the increase in plasma renin activity following a decrease in renal perfusion pressure due to supra-renal aortic constriction was completely inhibited by indomethacin. This suggested that prostaglandin synthesis was necessary for the increase in renin release following stimulation of the renal baroreceptor. These results were confirmed by Berl *et al*. who also demonstrated that prostaglandin synthesis inhibition prevented an increase in plasma renin activity following supra-renal aortic constriction (Berl, Henrich, Erickson and Schrier, 1979). These studies indicate that prostaglandins are involved in the baroreceptor mechanism of renin

release, but they give no indication as to which prostaglandins are important. In an anaesthetised uninephrectomised dog model, in which the remaining kidney was denervated and non-filtering, infusion of PGI_2, PGE_2 or 13,14-dihydro-PGE_2 into the renal artery resulted in an increase in renal blood flow and plasma renin activity (Gerber, Keller and Nies, 1979). PGI_2 was more potent than PGE_2 although the PGE_2 metabolite, 13,14-dihydro-PGE_2 was found to be most potent in increasing plasma renin activity. An intrarenal infusion of papaverine resulted in a similar increase in renal blood flow but had no effect on plasma renin activity indicating that prostaglandins were having an effect on renin release which was independent of their renal vasodilator activity. It would seem, however, that prostaglandins are only important in the renal baroreceptor mechanism of renin release when the drop in renal perfusion pressure is within the renal autoregulatory range. Blackshear *et al*. demonstrated that whilst indomethacin prevented an increase in renin release in response to a decrease in perfusion pressure which was within the autoregulatory range, if perfusion pressure was lowered such that it was accompanied by a decrease in renal blood flow, indomethacin failed to prevent an increase in renin release (Blackshear, Spielman, Knox and Romero, 1979). Thus whilst prostaglandins may be necessary for increased renin release upon stimulation of the renal baroreceptor by a moderate reduction in renal perfusion pressure, when perfusion pressure is lowered below the autoregulatory range, the renal baroreceptor and/or some secondary mechanism is capable of stimulating renin release independent from prostaglandin synthesis. This prostaglandin-independent mechanism of renin release in response to a large drop in perfusion pressure may explain why other investigators have failed to observe any effect of indomethacin on the increased plasma renin

activity following a 50% reduction in renal perfusion pressure in uninephrectomised renal denervated anaesthetised dogs (Seymour and Zehr, 1979).

Dietary sodium deprivation is associated with a decrease in early distal tubular sodium delivery which results in a macula densa mediated increase in renin release. Initial studies investigating the role of prostaglandins in the macula densa mechanism of renin release failed to demonstate any effect of indomethacin on the increase in plasma renin activity in response to sodium deprivation in either humans or conscious dogs (Norbiato, Bevilacqua, Raggi, Micossi, Moroui and Fasoli, 1978; Opgenorth, Wappel and Zehr, 1980). However, it was later demonstrated that if β-adrenergic activity was blocked with propranolol, indomethacin administration resulted in a decrease in plasma renin activity in both sodium depleted humans and conscious dogs (Frolich, Hollifield, Michelakis, Vesper, Wilson, Shand, Seyberth, Frolich and Oates, 1979; De Forrest *et al.*, 1980). This suggested that in addition to the macula densa mechanism of renin release, which may be mediated by prostaglandins, sympathetic nerve activity also contributed to the increased renin release during sodium deprivation by a mechanism independent of prostaglandin synthesis. In an anaesthetised, uninephrectomised dog model, in which the remaining kidney was denervated and infused with propranolol and the renal baroreceptor was inactivated by an infusion of papaverine, a supra-renal aortic constriction which reduced renal perfusion pressure by 50% resulted in a 90% reduction in urinary sodium excretion and an increase in plasma renin activity (Gerber, Nies and Olsen, 1981). Indomethacin or meclofenamate administration had no effect on the reduced sodium excretion in response to aortic constriction but prevented the associated increase in plasma renin activity. This would suggest that the macula densa

mechanism of renin release is mediated by prostaglandins. It should be noted, however, that this conclusion is dependent upon the assumption that the renal baroreceptor was inactivated. In the conscious rat, prior indomethacin administration partially inhibited an increase in plasma renin activity when the animals were subjected to dietary sodium deprivation (Suzuki *et al.*, 1981b). In a study using a micropuncture technique in anaesthetised rats, dietary sodium deprivation produced a decrease in early distal tubular delivery of sodium and an increase in plasma renin activity (Francisco, Osborne and Di Bona, 1982). This response occurred in a denervated kidney, when mean arterial pressure was constant within the renal autoregulatory range, renal blood flow was stable and renal perfusion pressure was not changing. This indicates that the increase in plasma renin activity was due to stimulation of the macula densa. Under the same conditions, indomethacin administration had no effect on the early distal tubular sodium delivery but inhibited the increase in plasma renin activity suggesting that prostaglandins were involved in mediating the increase in renin release following stimulation of the macula densa.

Intravenous infusion of isoprenaline into uninephrectomised renally denervated anaesthetised dogs results in an increase in plasma renin activity presumably as a consequence of direct stimulation of β receptors in the juxtaglomerular cells. Prior administration of indomethacin was found to have no effect on the increase in plasma renin activity in response to isoprenaline infusion suggesting that β-adrenergic stimulation of renin release is not mediated by prostaglandins (Berl *et al.*, 1979; Seymour and Zehr, 1979). In a superfused preparation of isolated rat glomeruli, isoprenaline increased the amount of renin released into the effluent without having any effect on the quantity of PGE_2 released

(Beierwaltes, Schryver, Olson and Romero, 1980). Prior inhibition of prostaglandin synthesis with meclofenamate had no effect on the ability of isoprenaline to increase renin release. Isoprenaline also increased renin release in the isolated perfused rabbit kidney (Vandogen, Tunney, Mahoney and Barden, 1981). In this preparation, the quantity of 6-keto-$PGF_{1\alpha}$ released by the kidney was not altered by isoprenaline and indomethacin had no effect on the renin response to isoprenaline. The results of these studies suggest that neither PGE_2 nor PGI_2 are involved in mediating the increase in renin release following β-adrenergic receptor stimulation with isoprenaline. Contrary to this, however, Campbell *et al*. demonstrated that in conscious rats, indomethacin could inhibit the increase in plasma renin activity due to reflex activation of the sympathetic system by hydrallazine, β-adrenergic receptor stimulation by isoprenaline and stimulation of a site distal to the β-adrenergic receptor by dibutyryl cyclic AMP (Campbell, Graham and Jackson, 1979). These results were supported by a similar study in conscious rats which demonstrated a partial inhibition of the renin response to isoprenaline by indomethacin (Suzuki *et al*., 1981b) and indicated a participation of prostaglandins in the release of renin following sympathetic stimulation. Kopp *et al*. showed that during low level renal nerve stimulation in the anaesthetised dog there was very little change in renal haemodynamics and the increase in plasma renin activity was mediated by β_1-adrenergic receptors. During high level renal nerve stimulation, the increase in plasma renin activity was partly mediated by β_1-adrenergic receptors but was also secondary to the α-adrenergic receptor mediated renal vasoconstriction (Kopp, Aurell, Nilson and Ablad, 1980). Indomethacin had no effect on the increase in plasma renin activity during low level renal nerve stimulation but partially inhibited the increase in plasma renin activity during high level

renal nerve stimulation (Kopp, Aurell, Sjölander and Ablad, 1981). The α-adrenergic antagonist, phenoxybenzamine had no additional effect to the indomethacin in preventing the increase in plasma renin activity. However, administration of the β_1-adrenergic receptor antagonist metoprolol in addition to the indomethacin resulted in the renin response to high level renal nerve stimulation being abolished. These results implied that the β_1-adrenergic receptor component of renin release following renal nerve stimulation did not require prostaglandin synthesis whilst the α-adrenergic receptor component, probably secondary to haemodynamic changes, was dependent upon prostaglandins to mediate renin release. Intrarenal infusion of the α-adrenergic receptor agonist, phenylephrine into anaesthetised dogs in which the β-adrenergic receptors were blocked with sotalol, resulted in a decrease in renal blood flow and sodium excretion and an increase in plasma renin activity (Olsen, Nies and Gerber, 1981). Indomethacin prevented the increase in plasma renin activity during phenylephrine infusion but had no effect on the decreased blood flow or sodium excretion. In this model, phenylephrine infusion had no effect on plasma renin activity if the kidney was rendered non-filtering, therefore it would seem that the phenylephrine-induced increase in renin release was secondary to stimulation of the macula densa which required prostaglandins to mediate the increase in renin release. Seymour *et al*. failed to observe an α-adrenergic receptor component in renin release during noradrenaline infusion in conscious dogs and rats and demonstrated that α-adrenergic receptor block with phentolamine potentiated the renin response to noradrenaline, suggesting that α-adrenergic receptor stimulation was inhibiting renin release (Seymour, Davis, Echtenkamp, Dietz and Freeman, 1981). Concentrations of isoprenaline and noradrenaline which had no effect on mean arterial pressure

increased plasma renin activity and this effect was potentiated by prior indomethacin administration. This potentiation of adrenergically mediated renin responses by indomethacin does not necessarily imply that prostaglandins were suppressing renin release but may result from the removal of an inhibitory effect of prostaglandins on post junctional adrenergic receptors (Frame, Hëdquist and Aström, 1974). It therefore remains controversial as to whether prostaglandins are involved in the renin response to sympathetic nerve stimulation. It seems likely that β-adrenergic receptor stimulation can increase renin release by a mechanism independent of prostaglandin synthesis although an α-adrenergic receptor mediated increase in renin release may require prostaglandin synthesis. There may also be species variation, with adrenergically mediated renin release being prostaglandin dependant in the rat but prostaglandin independent in other species.

Despite the large body of evidence implicating a role for prostaglandins in the control of renin release, their importance as regulators of renin release under physiological conditions and the extent to which they mediate the other intrinsic mechanisms of renin release remains the subject of debate.

1.5.2 Stimulation of prostaglandin synthesis by angiotensin II

An increase in renin release in response to a variety of stimuli results in an increase in angiotensin II formation which causes vasoconstriction and sodium retention both by a direct action and by stimulation of aldosterone secretion. Several lines of investigation have suggested that angiotensin II stimulates PGE_2 and PGI_2 synthesis which may serve to modulate the action of angiotensin II by means of their vasodilator and natriuretic actions.

Infusion of angiotensin II into the renal artery of anaesthetised dogs was demonstrated to result in the release of a PGE_2-like substance into the renal venous blood, as detected by a blood-bathed bioassay technique (McGiff, Crowshaw, Terragno and Lonigro, 1970). The release of this PGE_2-like substance in response to angiotensin II was shown to coincide with the recovery of renal blood flow (Aiken and Vane, 1973). Intravenous infusion of angiotensin II into humans was found to increase urinary PGE_2 excretion (Frolich *et al.*, 1975) and intrarenal infusion of angiotensin II in anaesthetised dogs resulted in an increase in PGE_2 excretion (Dunn, Liard and Dray, 1978). However, Blasingham and Nasjletti, using a similar model failed to observe an increase in urinary PGE_2 excretion during intravenous angiotensin II infusion (Blasingham and Nasjletti, 1980). An intravenous infusion of angiotesnin II into anaesthetised cats was found to increase the amount of PGI_2-like substance in arterial blood (Swies, Radomski and Gryglewski, 1979) and an intrarenal infusion of angiotensin II increased the amount of PGI_2-like substance in the arterial blood of anaesthetised dogs and rats (Shebuski and Aiken, 1980; Dusting and Mullins, 1980). That the increase in PGI_2 synthesis during angiotensin II infusion was not merely a result of mechanical stimulation due to the vasoconstriction was demonstrated by Mullane and Moncada who observed an increase in PGI_2-like substance in the venous blood of anaesthetised dogs when angiotensin II was infused either intravenously or intrarenally. However, no increase in PGI_2-like substance was observed during infusions of concentrations of adrenaline or 5-hydroxytryptamine which caused the same degree of vasoconstriction (Mullane and Moncada, 1980). In a recent study, however, no increase in immunoreactive 6-keto-$PGF_{1\alpha}$ was detected in the plasma of conscious rabbits during intravenous infusion of angiotensin

II although an increase in the plasma concentration of PGE_2 was detected (Nasjletti and Malik, 1982). This suggested that vascular PGI_2 synthesis was not stimulated by angiotensin II.

Isolated preparations have also been used to investigate the effects of angiotensin II on prostaglandin synthesis. Needleman *et al*. demonstrated that angiotensin II released a PGE_2-like substance when infused into the isolated rabbit kidney (Needleman, Kauffman, Douglas, Johnson and Marshall, 1973). The release of prostaglandin could be inhibited by the angiotensin II antagonist [8-cys-teine]-angiotensin II, suggesting that the stimulation of prostaglandin synthesis by angiotensin II was a specific receptor-mediated effect. Incubation of angiotensin II with whole cell preparations of rat renal papillae increased the PGE_2 content in the tissue and in the incubating medium (Danon, Chang, Sweetman, Nies and Oates, 1975). Angiotensin II also increased PGE_2 release from cultured rabbit reno-medullary interstitial cells, an effect which could be inhibited by the anti-malarial drug, mepacrine (Zusman and Keiser, 1977). This suggested that angiotensin II increased prostaglandin synthesis by activation of phospholipase resulting in an increase in arachidonic acid availability. Other investigators have failed to demonstrate any effect of angiotensin II on PGE_2 synthesis by isolated rabbit or rat renal medullary tissue (Sirois and Gagnon, 1974; Anees, Sweet, Tan and Mulrow, 1979). Silberbauer *et al*. observed that angiotensin II incubated with isolated sections of rat kidney resulted in an increase in PGI_2-like substance in the supernatant, as detected by its ability to prevent ADP-induced platelet aggregation (Silberbauer, Sinzinger and Winter, 1979). An increase in PGI_2 release was also demonstrated when angiotensin II was infused into the isolated rabbit kidney, although the increase in PGI_2 synthesis was much smaller than the increase in

PGE_2 synthesis (Needleman, Bronson, Wyche, Sivakoff and Nicolaou, 1978).

Angiotensin II has also been shown to increase prostaglandin synthesis in other tissues besides the kidney. PGE_2 and PGI_2 release from the isolated rabbit heart was increased during infusion of angiotensin II and contrary to the kidney, PGI_2 synthesis was stimulated to a greater extent than PGE_2 synthesis (Needleman, Marshall and Sobel, 1975; Needleman *et al.*, 1978). Cultured human vascular endothelial cells have been shown to release PGE_2 into the culture medium and incubation with angiotensin II increased the PGE_2 release (Gimbrone and Alexander, 1975). Isolated perfused rabbit mesenteric blood vessels released a PGE_2-like substance in response to angiotensin II (Blumberg, Denny, Marshall and Needleman, 1977) and isolated rat mesenteric vasculature has been shown to release PGE_2 and PGI_2 when perfused with angiotensin II (Nolan, Dusting and Martin, 1981; Dusting, Mullins and Nolan, 1981). Perfusion of mesenteric vasculature with angiotensin I also resulted in an increase in prostaglandin release although this release could be inhibited by an angiotensin I converting enzyme inhibitor. This suggests that angiotensin I requires to be converted to angiotensin II in order to stimulate prostaglandin synthesis (Blumberg *et al.*, 1977; Nolan *et al.*, 1981). Angiotensin II can also induce release of a PGI_2-like substance from perfused guinea-pig and rat lungs (Grodzinska and Gryglewski, 1980; Dusting *et al.*, 1981). In a recent study, angiotensin II had no effect on PGI_2 production by cultured porcine aortic endothelial cells (Whorton, Young, Data, Barchowsky and Kent, 1982).

It is apparent that although there is much in the literature to suggest that angiotensin II stimulates prostaglandin synthesis in a number of different tissues including the kidney, not all the experimental evidence

supports such an action of angiotensin II. It should be noted that in the majority of studies demonstrating an effect of angiotensin II on prostaglandin synthesis, pharmacological doses of angiotensin II were used. Such large concentrations may not represent the actions of angiotensin II under physiological conditions.

Inhibition of prostaglandin synthesis by indomethacin augments the renal vasoconstrictor action of angiotensin II in anaesthetised dogs and sheep (Aiken and Vane, 1973; Beilby *et al.*, 1981) and potentiates the pressor response to angiotensin II in sodium replete and sodium deplete humans (Negus, Tannen and Dunn, 1976; Speckart, Zia, Zisper andHorton, 1977). In the sodium replete anaesthetised dog, inhibition of prostaglandin synthesis with meclofenamate had no effect on systemic blood pressure or renal blood flow. If, however, angiotensin II was infused, meclofenamate administration resulted in an increase in systemic blood pressure and a decrease in renal blood flow (Blasingham and Nasjletti, 1980). In the sodium deplete anaesthetised dog, meclofenamate administration increased systemic blood pressure and decreased renal blood flow. This effect could be inhibited by prior administration of the angiotensin II antagonist, saralasin. Similarly, in conscious dogs, inhibition of prostaglandin synthesis with meclofenamate had no effect on renal blood flow unless the renin-angiotensin system was stimulated by sodium depletion whereupon meclofenamate administration resulted in a decrease in renal blood flow (Blasingham *et al.*, 1980). These results suggested that increased levels of angiotensin II, whether by exogenous infusion or due to sodium deprivation, resulted in increased synthesis of prostaglandins which acted to modify the effects of angiotensin II by causing a vasodilatation. However, Zimmerman failed to observe any effect of meclofenamate on renal blood flow in Goldblatt

hypertensive conscious dogs even though plasma renin activity was elevated (Zimmerman, 1978), suggesting that although angiotensin II formation was increased, prostaglandin synthesis had not been stimulated.

Several studies have attempted to demonstrate an increase in PGE_2 synthesis during stimulation of the renin-angiotensin system by sodium deprivation. Some investigators have observed an increase in renal venous and urinary PGE_2 levels during sodium deprivation (Oliver, Pinto, Sciacca and Cannon, 1980; Rathaus, Podjarny, Weiss, Ravid, Bauminger and Bernheim, 1980), whilst others have failed to observe any change in urinary PGE_2 levels during sodium depletion (Lifschitz, Patak, Raclem and Stein, 1978; Blasingham *et al.*, 1980). It therefore remains controversial as to whether increased formation of angiotensin II, following physiological stimulation of renin release, results in an increase in prostaglandin synthesis.

Studies demonstrating an effect of prostaglandins to stimulate renin release and studies demonstrating an effect of angiotensin II to stimulate prostaglandin synthesis afford two pieces of evidence which are difficult to reconcile within the same physiological mechanism. Angiotensin II has a direct negative feedback effect on the juxtaglomerular cells to inhibit renin release (Vander and Geelhoed, 1965; Bunag, Page and McCubbin, 1967). However, angiotensin II may also stimulate prostaglandin synthesis within the kidney which in turn could result in an increase in renin release constituting a positive feedback mechanism (Stahl, Ahmad, Attallah, Bloch and Lee 1979). It has been suggested (Attallah et al. 1982) that prostaglandins, by stimulation of renin release, have a pro-hypertensive action and, by causing vasodilation secondary to stimulation of synthesis by angiotensin II, an anti-hypertensive action. It seems likely that the degree of contribution of prostaglandins to these

mutually antagonistic mechanisms varies with experimental circumstances and therefore it is perhaps not surprising that agreement is lacking regarding the overall nature and extent of prostaglandin involvement in control of renal blood flow and systemic blood pressure. It is possible that under both experimental and physiological conditions, stimuli resulting in an increase in the prohypertensive prostaglandin-renin-angiotensin system are associated with an offsetting secondary increase in the antihypertensive prostaglandins. Conversely, a primary increase in antihypertensive prostaglandins may be countered by a secondary increase in the renin-angiotensin system. A delicate balance may therefore exist between these two important opposing renal humoral systems in which the final mechanisms regulating their positive feedback actions remain obscure.

Alternatively, the synthesis and actions of prostaglandins in the kidney may be compartmentalised such that a stimulus for renin release may be mediated by prostaglandins synthesised in or near the juxtaglomerular cells. Modulation of the actions of the consequently formed angiotensin II may occur by stimulation of prostaglandin synthesis in the renal vasculature which may not necessarily result in a further increase in renin release.

It is apparent that there is a complex interaction between prostaglandins and the renin-angiotensin system and that further investigation is required to elucidate the precise nature of this interaction. Most work in this field has focussed attention on PGE_2. However, more recent studies have suggested that PGI_2 may be more integral in the relationship between prostaglandins and the renin-angiotensin system. It is the aim of this thesis to attempt to further clarify the relationship between PGI_2 and the renin-angiotensin system.

1.6 Choice of Animal Model

In choosing an animal model, it was important to ensure that the renin-angiotensin system and the prostaglandin system were operating under as physiological conditions as experimental design would allow. It was also thought pertinent to choose an animal species in which the responses to the renin-angiotensin system and renal prostaglandins showed an analogy to the human situation.

Several studies have demonstrated that anaesthetic drugs, when used in doses which produce a surgical plane of anaesthesia, stimulate the release of renin in man and experimental animals (Kaldor, Gachalyi, Kallau, Fekete and Foldrari, 1975; Johnson and Malvin, 1975; Yun, Kelly, Bartter and Tate, 1978). The mechanisms by which anaesthetic agents release renin appear to be as diverse as the pharmacological properties of the anaesthetics themselves with each anaesthetic agent apparently altering renin by a different mechanism (for review, see Keeton and Campbell, 1980). It is therefore an obvious disadvantage to investigate the effects of drugs on the renin-angiotensin system in an animal which has been administered with an anaesthetic agent since this will have resulted in prior stimulation of renin release.

Although anaesthesia itself has no effect on basal prostaglandin levels, laparotomy performed under anaesthesia markedly elevates prostaglandin levels (Terragno *et al.*, 1978). Under these conditions of acute surgical stress renal venous PGE_2 levels in the dog and urinary PGE_2 and $PGF_{2\alpha}$ levels in the rat are significantly higher than those found in conscious animals (Terragno *et al.*, 1978; Scherer, Schnermann, Sofroniev and Weber, 1978).

It was therefore apparent that an anaesthetised surgically stressed animal did not represent a suitable model in which to investigate the relationship between prostaglandins and the renin-angiotensin system and all studies in this thesis were performed in conscious dogs.

The dog was favoured to other species for three main reasons. Firstly, the larger anatomical size of the dog compared to rat or rabbit was more practical for the insertion and maintenance of indwelling catheters. Secondly, previous experience in this laboratory had demonstrated that the foxhound breed of dog had a docile temprement well suited to chronic work and could be trained to sit in a quiet room for several hours without becoming overstressed. Thirdly, as far as can be ascertained from the literature, the dog provides a good analogy with the human as far as the renin-angiotensin system and the renal prostaglandin systems are concerned. The suitability of the rat as an animal model for the study of renal prostaglandins has been questioned since the renal vascular response to PGE_2 in the rat differs from that in other species. PGE_2 results in renal vasodilation in the dog (Vander, 1968) and rabbit (Malik and McGiff, 1975), whilst in the rat, although low doses of PGE_2 result in renal vasodilatation (Haylor & Towers, 1982; Jackson et al 1982) larger doses of PGE_2 may result in renal vasoconstriction (Baer and Mc Giff, 1979).

1.7 Manipulation of the Renin-Angiotensin System

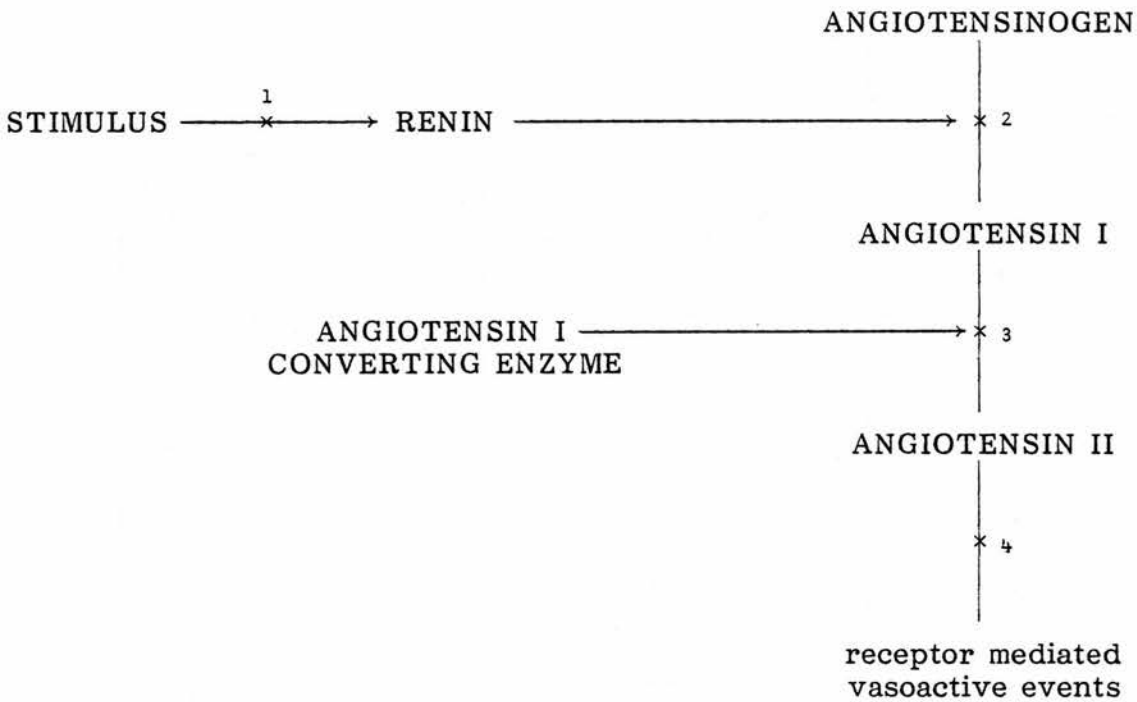
1.7.1 Physiological stimulation of renin release

Brown *et al.* discovered that sodium depletion in humans elevated plasma renin activity and that sodium loading suppressed plasma renin activity (Brown, Davies, Lever and Robertson, 1963). This observation has been corroborated by many investigators in both man and experimental animals (Romero, Staneloni, Dufan, Dohmen, Binia, Kliman and Fasciolo, 1968; Brubacher and Vander, 1968; Keeton, Pettinger and Campbell, 1976). During dietary sodium deprivation, total daily sodium excretion falls to very low levels due to a decrease in the fractional excretion of the filtered sodium load. Much of the increase in the efficiency of sodium reabsorption occurs in the proximal tubule (Mohammed, Di Scala and Stein, 1974), therefore the tubular sodium concentration and sodium load passing the macula densa segment of the distal tubule will be decreased. This decrease in sodium transport to the macula densa results in stimulation of renin release and the consequently formed angiotensin II further conserves sodium both by a direct action and by stimulation of aldosterone secretion.

Sodium depletion in experimental animals is achieved by initial administration of frusemide followed by maintenance on a sodium free diet. This procedure is routinely used in laboratories to physiologically stimulate the renin-angiotensin system.

1.7.2 Pharmacologic interruption of the renin-angiotensin system

The renin-angiotensin system offers a number of sites which could be selectively inhibited (Figure 1.4). Renin is released from the kidney by such a variety of stimuli that it would be difficult to completely block renin release with one agent. Under certain conditions, renin release



1 β-adrenergic receptor blocking drugs
Non-steroidal anti-inflammatory drugs, e.g. indomethacin.

2 Pepstatin
Renin antibodies
Substrate analogues.

3 Teprotide
Captopril.

4 Angiotensin II analogues, e.g. saralasin.

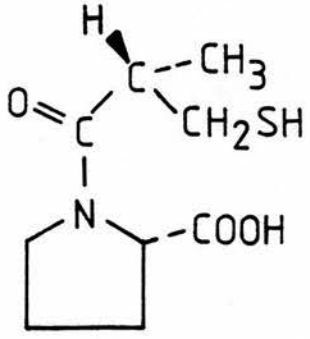
FIGURE 1.4: Pharmacologic interruption of the renin-angiotensin system.

can be at least partly inhibited by indomethacin (Frolich *et al.*, 1975) and by β-adrenergic receptor blocking drugs such as propranolol (Michelakis and McAllister, 1972). Following release, renin cleaves the leucyl-leucine bond that joins the amino-terminal decapeptide angiotensin I to the remainder of the substrate, angiotensinogen which is synthesised in the liver. The action of renin on angiotensinogen has been antagonised by nonspecific proteases such as pepstatin (Gross, Lazar and Orth, 1972) by antibody to renin (Romero, Hoobler, Kozak and Warzynski, 1973) and more specifically by structural analogues of the renin substate (Paulsen, Burton and Haber, 1973). The decapeptide angiotensin I is converted within the vascular bed of the lungs, kidneys and other organs to the octapeptide angiotensin II by hydrolytic removal of its C-terminal, his-leu dipeptide residue. This conversion is catalysed by a membrane bound exopeptidase, a peptidyl carboxyhydrolase known as angiotensin I converting enzyme or kininase II, the same enzyme being responsible for the hydrolytic removal of the C-terminal dipeptide from bradykinin. The formation of angiotensin II from angiotensin I provides two further sites where the renin-angiotensin system could be inhibited, the inhibition of the converting enzyme and antagonism of the vasoactive angiotensin II at the receptor.

The first group of compounds which offered any degree of specificity and which were used extensively in research were angiotensin II analogues found to have antagonist activity. Marshall *et al.* demonstrated that 4-Phe-8-Tyr-angiotensin II was a competitive receptor antagonist of angiotensin II (Marshall, Vine and Needleman, 1970). Other analogues of angiotensin II with aliphatic amino acids (alanine, isoleucine and glycine) substituted for the phenylalanine residue in the 8-position of angiotensin II were synthesised in other laboratories (Pals, Masucci,

Sidpos and Denning, 1971; Turker, Hall, Yamamoto, Sweet and Bumpus, 1972). In addition, in most of these compounds the 1 position was occupied by the non-mammalian amino-acid sarcosine since this substitution was found to prolong the half-life of the peptides and thereby increase their potency. The most extensively characterised receptor antagonist of angiotensin II is 1-Sar-8-Ala-angiotensin II, also known as saralasin. Saralasin has been used extensively to investigate the physiological and pathophysiological role of angiotensin II (Agabiti-Rosei, Brown, Brown, Fraser, Trust, Lever, Morton and Robertson, 1979; Fagard, Amery, Lijnen and Reybrouck, 1977; North, Tan and Mulrow, 1977). However, saralasin and other angiotensin analogues are not devoid of intrinsic agonist angiotensin-like activity, therefore caution is required in the interpretation of the results (Anderson, Streeton and Dalakos, 1977; Hollenberg, Williams, Burger, Ishikawa and Adams, 1976; McGregor and Davies, 1976).

The first inhibitors of angiotensin I converting enzyme which were potent and specific enough for extensive study were a series of peptides found in the venom of the Brazilian viper, *Bothrops jararaca*. A crude mixture of these peptides was first described by Ferreira as 'bradykinin-potentiating factor' (Ferreira, 1965), but the peptides were also found to be potent inhibitors of angiotensin I converting enzyme (Bakhle, 1968). Several peptides were isolated from the snake venom (Ferreira, Bartlett and Greene, 1970; Ondetti, Williams, Sabo, Plusac., Weaver and Kocy, 1971), but the most thoroughly studied one was a nonapeptide (Pry-Trip-Pro-Arg-Pro-Gln-Ile-Pro-Pro), the synthetic form of which is designated SQ20881 or teprotide (Glu-Trip-Pro-Arg-Pro-Gln-Ile-Pro-Pro). Teprotide and other similar peptides compete with angiotensin I for the converting enzyme, thereby inhibiting the conversion of angio-



CAPTOPRIL (mol wt 217)

(N-[(25)-3-Mercapto-2-methylpropionyl]-l-proline)

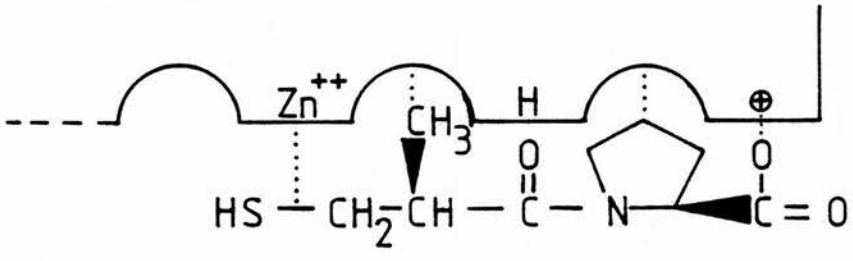


FIGURE 1.5: The binding of captopril to angiotensin I converting enzyme.

tensin I to angiotensin II. Although teprotide was effective in inhibiting the effects of angiotensin I both on systemic blood pressure and renal function (Kimbrough, Vaughan, Carey and Ayers, 1977; Niarchos, Pickering, Case, Sullivan and Laragh, 1979), it did not have wide clinical application since it was liable to enzymatic degradation and therefore had to be administered intravenously. A programme of extensive structure-activity studies was therefore initiated by Ondetti *et al*. to develop an orally active inhibitor of angiotensin I converting enzyme. D-3-mercapto-2-methylpropanoyl-1-proline, designated SQ14225 or captopril (Figure 1.5) was found to be the most potent orally active inhibitor of angiotensin I converting enzyme (Cushman, Cheung, Sabo and Ondetti, 1977; Ondetti, Rubin and Cushman, 1977). The salient features of captopril with respect to binding to angiotensin converting enzyme are (a) binding of the sulphydryl to zinc, (b) binding of the proline carboxylic acid to a cationic site on the enzyme and (c) incorporation of the amino acid proline, and a methyl side chain adjacent to proline, since the amino acid increased affinity for the enzyme and the methyl group was thought to increase activity by restricting the conformation of the molecule. Captopril, like teprotide, is a competitive inhibitor of angiotensin I converting enzyme and has a half-life of approximately three hours. Captopril has been studied extensively and has been found to be an effective anti-hypertensive agent in both clinical and laboratory situations (Morton, Tree, Casals-Stenzel, 1982; Gavras, Brunner and Turin, 1978). There has been controversy over the precise mechanism of the anti-hypertensive action of captopril since elevated bradykinin levels may be contributing to the hypotension in addition to inhibition of angiotensin II formation. Some lines of investigation have also implicated prostaglandins as a contributing factor towards the hypotension following

captopril administration. The controversy surrounding the mechanism of action of captopril will be discussed fully in Section IV.

1.8 Assessment of Renal Function

1.8.1 Renal blood flow

The major problem commonly encountered in conscious animal work is that of recurrent infection, the most common cause of which is due to indwelling catheters. For this reason it was thought preferable to use as little chronic instrumentation in the dog models as possible. Indwelling venous catheters were necessary for infusion of drugs and fluids but a non-invasive method was used to measure renal blood flow. Although non-invasive methods of measuring renal blood flow are less accurate than the use of flow probes, the problems known to be encountered during the use of indwelling flow probes in conscious animals were such that they were not regarded to be a practical alternative to a non-invasive method.

Non-invasive measurement of renal blood flow is based on the clearance of substances extracted almost completely by the kidney. A substance can be envisaged whose uptake by the kidney from renal arterial plasma by glomerular filtration and tubular secretion is complete such that none appears in the venous plasma. This imaginary substance would have an extraction efficiency E of 100%. The amount that appears in the urine per minute ($U \times \dot{V}$) equals the amount in the renal arterial plasma, $U \times \dot{V} = C \times P$, where C is the clearance of the hypothetical substance, and P is the plasma concentration of the substance in mg/100 ml. The first attempts to measure renal plasma flow used hippuran and phenol red (Chassis, Ranges, Goldring and Smith, 1938). The extraction

efficiency of these compounds was between 80 and 95%. It was later found that p-amino hippuric acid (PAH) was easier to measure than hippuran (Smith, Finklestein, Aliminosa, Cranford and Graber, 1945) and PAH which has been used to measure renal plasma flow for many years is employed in the work for this thesis.

In using PAH to measure renal plasma flow, it is assumed that plasma perfuses tubules which are uniformly efficient in extracting PAH. However, in various species the extraction efficiency of the juxtamedullary nephrons is substantially lower than that of the cortical nephrons. It should be noted that the extraction of PAH depends on a specific carrier whose activity is theoretically saturable at high plasma concentrations of PAH. However, clearance is not significantly depressed with the technique usually employed.

In practice, PAH is given with a loading dose followed by a continuous intravenous infusion. A steady state is achieved 30 min - 2 hr after the start of the infusion. To establish an appropriate plasma concentration of PAH, an injection of 8 mg/kg should be given followed by the establishment of a constant intravenous infusion of PAH at a rate calculated to approximately equal or slightly exceed the estimated rate of renal clearance. In all renal clearance studies, an important consideration is the accurate collection of timed urine collections which requires that urine is collected via an indwelling catheter. Urine collection periods should not be of less than 20 min duration, and plasma samples are taken at the end of each urine collection.

To determine the average plasma concentration of a solute (P) over a number of time intervals, a graphical interpolation can be performed. Successive plasma concentrations (P_1, P_2, P_3 etc) are plotted on semi-log graph paper against elapsed time, taking zero time either as the time at

which priming injection is administered or as the time at which the bladder is emptied and the collection of urine is begun. The exact value of P appropriate to each successive urine collection period is determined by interpolating to the value at the mid-point of the urine collection period.

Perhaps the most apparent disadvantage of measuring renal plasma flow by PAH clearance is that this method requires urine collections of at least 20 min and the value for PAH represents a mean value for renal plasma flow during that time. It may conceal fluctuations in the actual plasma flow rate that might occur during that time and give a false impression of the stability of renal plasma flow.

1.8.2 Glomerular filtration rate

A clearance study has also been employed for the measurement of glomerular filtration rate. A substance used in the assessment of glomerular filtration rate should be (a) metabolically inert and excreted exclusively by glomerular filtration, i.e. neither reabsorbed nor excreted by the tubules, (b) freely filterable through the glomerular capillary membranes, i.e. neither protein bound nor large enough to be held back in the process of ultrafiltration. The fructose polysaccharide inulin fulfils these criteria and the renal plasma clearance of inulin is generally accepted as the standard reference for glomerular filtration rate in all vertebrates (Shannon and Smith, 1935). Several other substances have been examined as substitutes for inulin including endogenous creatinine which is continuously introduced at a constant rate into the blood stream. Clearance of endogenous creatinine is the most extensively used procedure for the assessment of glomerular filtration rate in clinical work but it is generally accepted to be less accurate than inulin and this thesis has employed inulin clearance as a measure of glomerular filtration rate.

As with PAH, inulin is given as a loading dose followed by a continuous intravenous infusion, a steady state being achieved 30 min - 1 hr after the start of the infusion. An appropriate plasma concentration of inulin is achieved by injection of 50-100 mg/kg followed by the establishment of an intravenous infusion of inulin at a rate calculated to slightly exceed the estimated rate of renal clearance. Accurate urine collections should be of at least 30 min duration, plasma samples being taken at the end of each collection period. The average plasma concentration of inulin for each collection period is estimated as for PAH.

Similar to the measurement of renal plasma flow by PAH, the obvious disadvantage in the measurement of glomerular filtration rate by the clearance of inulin is that the value for inulin represents a mean value for glomerular filtration rate during each 30 min collection period. This may conceal any fluctuations in glomerular filtration rate which occur during that time.

1.8.3 Renin release

The term 'renin release' indicates the movement of renin molecules from the granular juxtaglomerular cells of the kidney into the blood flowing through the afferent glomerular arteriole. By strictest definition, 'renin secretion' is the minute output of renin by the kidney into the renal vein and is calculated by multiplying the renal plasma flow times the renal vein plasma renin activity minus the aortic plasma renin activity. However, it is not always possible, particularly in conscious animal work, to obtain renal venous blood and arterial plasma renin activity is taken to be indicative of renin secretion. Plasma renin activity is a measure of the ability of plasma to generate angiotensin I *in vitro* given the amount of renin and renin substrate present in the plasma

sample. Plasma renin activity is usually expressed as ng angiotensin I generated per ml of plasma per hr of incubation at 37°C (ng/ml/hr) and is generally accepted as being a reflection of the level of activity of the renin-angiotensin system *in vivo*.

1.9 Identification and Quantification of Prostaglandins

The extremely small amounts of prostaglandins normally present in biological samples necessitates the use of highly sensitive and specific methods for their analysis.

Biological assay methods have been used extensively for the detection and estimation of prostaglandins and thromboxanes. The advantages are good sensitivity together with specificity for biologically active compounds. Specificity can be enhanced by prior chromatographic procedures, by the use of pharmacological antagonists to compounds other than prostaglandins, and by assaying the sample on more than one type of tissue preparation. Bioassay has proved to be invaluable in the detection of new biologically active compounds and it was on such a system that Vane *et al.* detected PGI_2 (Moncada, Gryglewski, Bunting and Vane, 1976). However, together with the obvious disadvantage that bioassay cannot detect inactive metabolites, other analytical techniques have been demonstrated to have a higher sensitivity and specificity. The instability of PGI_2 makes it impossible to quantify except by bioassay, but other techniques have been developed to quantify 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, with the assumption that the concentration of 6-keto-$PGF_{1\alpha}$ is directly related to the concentration of PGI_2.

Radioimmunoassay (RIA) offers good specificity and is capable of measuring prostaglandins and their metabolites in the nanogram and

picogram range. This method can be applied to any prostaglandin provided that approximately 10 mg of the pure compound is available for the initial raising of the antibody. The unavailability of standards is a limitation to the use of RIA for the analysis of certain metabolites. Although RIA is time-consuming to set up, this disadvantage is compensated for by the speed of analysis allowed by the established technique. Cross-reactivity may cause problems when analysing structurally similar compounds, e.g. $PGF_{2\alpha}$ and $PGF_{1\alpha}$. However, these difficulties can be overcome by prior chromatographic separation of the interfering compounds. The specificity of an RIA method must be thoroughly confirmed by cross-reactivity, accuracy and precision tests as the technique will not provide evidence of interference in any individual sample. Ideally, the accuracy of RIA results should be confirmed by another analytical method, e.g. gas chromatography-mass spectrometry.

The problem of interference in an individual sample is eliminated when a prostaglandin is quantified by gas chromatography-mass spectrometry (GC-MS) since the analyst can observe a characteristically shaped ion current response and scrutinise each individual chromatogram for interfering substances. The ability of GC-MS to produce a 'fingerprint' of a molecule together with the obvious advantage of high precision when used for quantification, has resulted in the technique being used extensively for the identification, structural elucidation and quantification of prostaglandins and their metabolites.

The nature of the various polar functional groups in prostaglandins and their metabolites necessitates sample derivitisation prior to gas chromatography in order to achieve thermal stability, volatility and hence good gas chromatographic properties. The carboxylic acid group is rendered less polar by conversion to an ester, most commonly the

methyl ester. A number of derivatives have been employed to protect the hydroxyl groups, the most common of which is the trimethylsilyl (TMS) ether (Luukainer, Vanden-Neuvel, Haahti and Horning, 1961). A free ketone in the ω-side chain of a prostaglandin need not be derivitised, however the 6-ketol moiety of 6-keto-$PGF_{1\alpha}$ is particularly labile and must be stabilised by conversion of the ketone to an oxime (Green, 1969).

Following gas chromatography, the column eluant is introduced into the mass spectrometer via a separator which removes most of the carrier gas (Figure 1.6). In the ion source, ionisation of the sample molecules occurs by bombardment with electrons of a certain energy, generated from a rhenium filament and accelerated by a trap electrode. This bombardment with electrons will ionise and fragment the sample molecules. The per cent ionisation and extent of fragmentation depends, in part, on the constitution of the molecule, on the electron energy, and on the temperature of the ion source. The positively charged molecular ions and fragment ions resulting from ionisation are accelerated into the mass analyser. A portion of the ion beam (10%) is directed toward an electrode, and its output signal results in a tracing referred to as the total ion current. This indicates the optimum time to obtain a complete mass spectrum. The analyser tube is curved and has a magnet at its curvature. In order to obtain a complete mass spectrum, the magnetic field is continually increased while a constant amount of sample molecules are entering the ion source. This causes ions of increasing mass to impinge on the electron multiplier which is positioned behind an exit slit. The relationship between the mass of the ion (m), its charge (e, usually 1), the magnetic field strength (H) and the accelerating voltage (V) is given by:

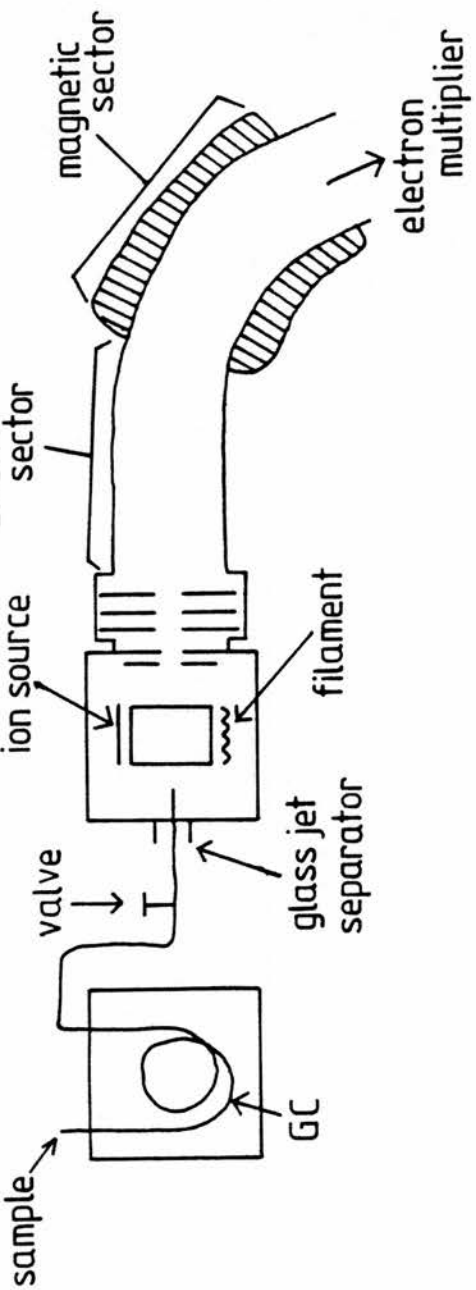


FIGURE 1.6: Diagrammatic representation of a gas chromatograph-mass spectrometer.

$$m^+/e = R^2 H^2 / 2V$$

where R is the radius of the curvature of the analyser tube (a constant). If V is held constant, then at a particular value of H, only one specific ion of m/e will pass through the exit slit to reach the electron multiplier. By continuously changing H, one ion after another is brought into focus and its abundance is registered. The resulting mass spectrum provides a 'fingerprint' for each compound and together with the chromatographic retention time of the derivative provides a highly specific method of identification.

In addition to the use of GC-MS to obtain a mass spectrum, it can be used in the multiple ion detection (also called selected ion monitoring) mode. The mass spectrometer is set to monitor only ion fragments of interest. Usually these are of high relative abundance in the mass spectrum (to confer sensitivity) and of high mass (to confer selectivity). Throughout a GC run, the ions are focussed alternately on the detector and their intensities are recorded separately as single ion chromatograms. This method of detection offers much higher sensitivity than recording the whole spectrum of ions and an additional advantage is that individual components may be monitored over the complete time of elution of a GC peak as distinct from a few milliseconds per ion when a mass spectrum is recorded. In the multiple ion detection mode, the mass spectrometer can be used quantitatively. This technique employs monitoring the response of the sample relative to an internal reference standard and comparing the response to a set of analytical calibration standards containing the same fixed amount of internal reference standard. The reference internal standard chosen is usually an analogue of the compound to be analysed and ideally is a stable isotope of the compound. These

stable isotopes are normally formed by replacing selected hydrogen atoms by deuterium in various positions of the molecule (for prostaglandins, d_4 compounds containing two deuteriums on C-3 and C-4 are normally used). A fixed amount of internal reference standard is added to each sample at the earliest possible stage in the analytical sequence, usually before the first extraction step and can therefore compensate for varying efficiencies during extraction, purification and derivatisation. A stable isotope internal reference standard and unlabelled standard compound will have virtually identical chromatographic characteristics and will not be distinguished until final detection by the mass spectrometer. At this stage, the instrument is set to record the selected ion chromatograms at the corresponding m^+/e value of the labelled and unlabelled compounds. The ions chosen are ones in which the deuterium atoms in the internal standard are retained in the ion fragment. A calibration curve may then be prepared by plotting the peak response ratio of the standard/internal reference standard against the amount of standard in the calibration standards and a comparison of the peak height ratio of samples enables their quantification.

The sensitivity of the method (generally low nanograms) depends on the cleanliness and the nature of the derivatised sample and normally extensive chromatography is required prior to quantification by GC-MS.

GC-MS used in the multiple ion detection mode provides the most specific assay method for the quantitative estimation of small amounts of lipids present in complex biological mixtures and this technique has been employed in subsequent sections of the thesis for the estimation of 6-keto-$PGF_{1\alpha}$ in urine.

SECTION II

General Methods

2.1 Preparation of the Conscious Animal Model

In all studies detailed in this thesis, conscious foxhound dogs were used. Eight male foxhounds weighing between 18 and 24 kg were surgically prepared and the same eight animals used in all studies.

2.1.1 Pre-operative procedures

On the day of surgery, animals were premedicated with chlorpromazine (Largactil, May & Baker, 20 mg i.v.) and anaesthesia was induced with thiopentone (Pentothal, May & Baker UK, 0.5 mg/kg). The areas to be operated upon were clipped and loose hair was removed. The animal was secured to the operating table and an endotracheal tube was inserted. A suitable depth of anaesthesia was maintained by administration of a mixture of oxygen : nitrous oxide : halothane (Fluothane, May & Baker UK). The shaved skin was swabbed with three antiseptic solutions:

1. Chloros (agricultural) diluted 1 : 10 with water.
2. Chlorhexidine (Hibitane, ICI, UK) diluted 1 : 10 with ethanol.
3. Thiomersal solution: 0.1% w/v thiomersal in 50% ethanol, 25% acetone and 25% water.

Thiomersal solution was also applied to the skin immediately prior to making any incisions.

All surgical instruments were sterilised by autoclave, and those persons partaking in the operative procedure scrubbed with Betadine surgical solution and wore sterile gowns, masks, hats and gloves.

2.1.2 Operative and post-operative procedures

(a) Formation of an isolated carotid artery loop

Mid and lateral incisions of 10-15 cm were made in the neck and blunt dissection used to expose the common carotid artery. Small vessels were ligated with either black silk (Surgisilk 4(1)) or thin catgut (DW202 Chromic 3) ties to prevent bleeding. The superior thyroid artery was ligated and the isolated carotid artery was cleared of adventitia as far as the bifurcation of the carotid artery. The skin between the two incisions was separated from the muscle layer, care being taken to maintain an adequate blood supply. The skin was then folded over the isolated section of the carotid artery and sutured with polyamide (Blue polyamide 2) to form a loop. The incision on the neck was sutured in a similar way and the wounds irrigated with thiomersal solution. An elastic bandage was secured around the animal's neck and pethidine (Roche, UK, 25 mg i.m.) administered immediately following surgery and again six hours later.

Animals were maintained on Septrin (Roche, UK) for five days after the operation, to reduce the risk of post-operative infection. The morning after the operation, the arterial loop was gently separated from the neck wound and cleaned with a sterile swab. A plaster bandage was then applied around the neck to prevent the animal from scratching the wound. The plaster was changed every three days, when the wounds were separated and swabbed with 1% cetrimide solution (Cetavlon, ICI, UK). After ten days, the sutures were removed and the plaster bandage replaced with a material collar until the wound was sufficiently healed, whereupon no further protection was required. Each animal was allowed one month to recover from the operation before any further surgical procedures were undertaken.

(b) Insertion of an indwelling venous catheter

A silastic catheter (Esco Ltd, UK, 1.0 mm i.d., 2.0 mm o.d., 70 cm long) was introduced at the back of the neck and led subcutaneously down the back to the flank by a sterile metal rod. An incision was made in the underside of a hind limb to expose the femoral vein, and the catheter led to this site. A branch of the femoral vein was cannulated and the catheter inserted and positioned in the inferior vena cava via the femoral vein. The catheter was then secured and its patency confirmed by flushing with sterile saline (sodium chloride injection B.P. 0.9% w/v, Steriflex, Boots Co., UK; heparin sodium 1000 u/ml, Weddel Pharmaceuticals Ltd, UK). The incision in the hind limb was then sutured with black silk. At the back of the neck, the catheter terminated in a metal lever fitting, a one-way metal tap and a metal obturator, and this was secured to a gauze collar. Pethidine (25 mg i.m.) was administered on completion of surgery and again six hours later.

Animals were maintained on penicillin (Crystamycin, Glaxo, UK, 0.5 mega unit/day) for five days to reduce the risk of post-operative infection. A material collar secured around the neck prevented the animal from interfering with the catheter. Every second day, after removal of dead space, the catheter was flushed with sterile saline and filled with heparinised saline (1000 u/ml). Animals were allowed seven days to recover from this operative procedure before being incorporated into an experimental regime.

2.2 Treatment of Infection

Rectal temperature and body weight were recorded daily and a chart kept. Pyrexia and loss of appetite were taken to indicate infection and this was treated by administration of penicillin (0.5 mega unit/day i.m.) for five days. Animals were removed from experimental studies during this period. In the majority of cases, this antibiotic regime was found to be successful. However, prolonged infection or interference by the animal sometimes necessitated removal of the catheter. Catheter patency was normally maintained for up to four weeks which meant that each animal had at least two catheters inserted in order to complete the series of studies.

2.3 Diet

All animals were fed a standard diet containing 50 mMoles sodium and 40 mMoles potassium per day. The diet was supplemented with ferrous sulphate (Laporte, UK, 200 mg/day).

Sodium depletion was induced by frusemide (Hoechst Ag., 200 mg i.v.) and maintained by a diet containing less than 10 mMoles sodium/day. Potassium chloride (slow K, Ciba UK) was given to maintain a daily potassium intake of 40 mMoles. At the end of each sodium deplete period, animals were housed in metabolic kennels and two 24-hour urine collections taken to allow determination of sodium excretion. In all cases, sodium excretion was found to be less than 8 mMoles/day indicating that animals were maximally retaining sodium.

2.4 Experimental Procedures

All studies were performed in a quiet room where animals had been trained to sit or stand on a table, restrained by a collar and lead such that they could turn through 90° but not 180°. The exact protocols for the various studies are described in detail in the following sections, however basic experimental procedures were common to all studies.

2.4.1 Administration of fluids and drugs

Intravenous infusions of fluids and drugs were made via the indwelling venous catheter. All administrative tubing was sterile and where possible, disposable sterile anaesthetic and y-junction extension sets (Travenol Laboratories Ltd, UK) were used. Where disposable tubing could not be used, sterility was achieved by storage in aqueous hibitane. Tubing was connected from the pumps to the animal such that three different solutions could be infused simultaneously (Figure 2.1). Modest water diuresis was established by infusion of sterile dextrose solution (Dextrose solution 5% w/v, Steriflex, Boots Co., UK), the infusion rate being controlled by a Watson-Marlow roller pump. Infusion of drug solutions was achieved by the use of a Harvard dual-syringe infusion pump. Drug solutions were sterilised by passing them through disposable sterile millipore filters (Millipore SA, France, 0.22 μM pore size).

2.4.2 Measurement of systemic blood pressure

To monitor systemic blood pressure, the isolated arterial loop was shaved of hair and swabbed with alcoholic Hibitane. Local anaesthesia was induced with 0.25 ml lignocaine injected subcutaneously at either end of the loop. The carotid artery was then cannulated with a 19 gauge butterfly needle. The needle was carefully positioned in the

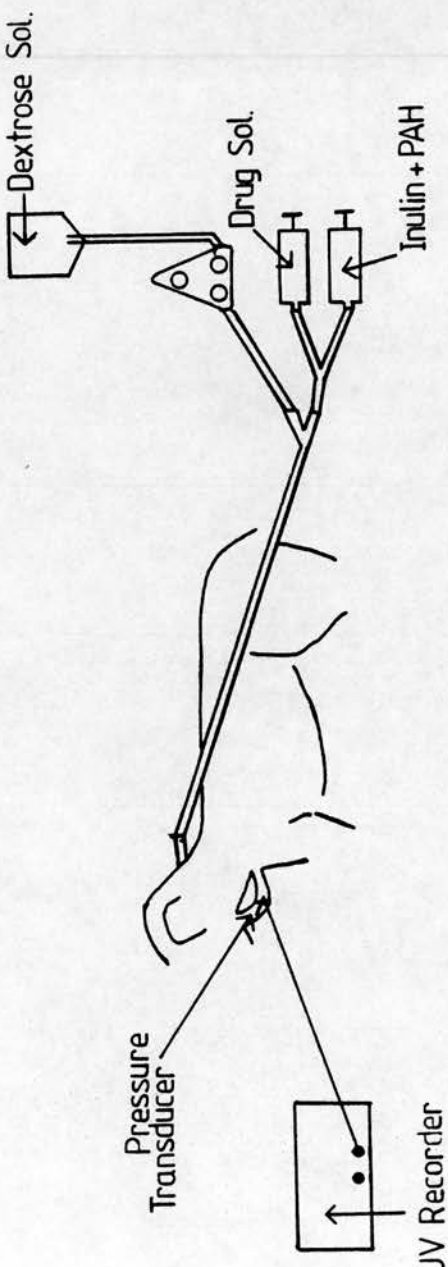


FIGURE 2.1: Diagrammatic representation of animal instrumentation.

vessel and kept in position with elastoplast. The catheter was connected to a portable pressure transducer (Statham P39) and the arterial line flushed with sterile saline and filled with heparinised saline (100 u/ml). The pressure transducer, which was secured to the animal's collar, was connected to a Honeywell recorder and a permanent record of systemic blood pressure kept on ultra-violet light sensitive paper. During every 30 min. recording period, mean arterial pressure was recorded for 25 min. and pulsatile pressure, from which heart rate was derived, was recorded for 5 min. Prior to the commencement of each study, the pressure recording system was calibrated from 0 to 100 mm Hg using a mercury manometer.

2.4.3 Collection of blood and urine samples

After removal of the dead space, blood samples of up to 25 ml were withdrawn from the arterial catheter. The blood was immediately put into pre-cooled lithium-heparin tubes and kept on ice. The arterial catheter was flushed with a small volume of sterile saline and filled with sterile heparinised saline (100 u/ml). Blood samples were centrifuged within 20 min, at 4°C, 2000 g for 25 min. The plasma was then aspirated and stored at -20°C until the time of assay.

Timed urine collections were made via a sterile catheter inserted into the bladder. Urine was therefore collected continuously with complete bladder emptying at the end of each collection period being achieved by manual palpation. This allowed accurate urine flow rates to be determined. Urine samples were stored at -20°C until the time of assay.

2.5 Estimations Performed on Plasma and Urine Samples

2.5.1 Estimation of renal plasma flow

After an initial loading dose of 8 mg/kg p-aminohippuric acid (PAH) (20% aminohippurate sodium, Merck, Sharp and Dohme, USA) diluted in sterile dextrose, was infused intravenously to maintain a plasma concentration of approximately 3 mg/100 ml.

The concentration of PAH in the plasma and urine samples was estimated by spectrophotometry using a continuous flow auto-analyser system (Technicon Auto-Analyser System AAI) (Harvey and Brothers, 1962). In brief, the PAH in the plasma or urine was diazotised with nitrite, and excess nitrite was destroyed with ammonium sulphate. The diazotised PAH was coupled with N(1-napthyl)-ethylene diamine dihydrochloride to produce the colour which was estimated in a colorimeter at 550 nm and recorded on a chart recorder. The colour produced in the plasma and urine samples was compared with that produced by standard solutions.

Renal plasma flow, expressed as ml/min, was taken to be PAH clearance (C_{PAH}) and was calculated by:

$$C_{PAH} = \frac{U_{PAH}}{P_{PAH}} \times \dot{V}$$

where U_{PAH} = concentration of PAH in the urine (mg %)
P_{PAH} = concentration of PAH in the plasma (mg %)
$\dot{V}$ = urine flow (ml/min)

2.5.2 Estimation of glomerular filtration rate

After an initial loading dose of 50-100 mg/kg, inulin (10% w/v, T. Kerfoot & Co., UK) diluted with sterile dextrose, was infused intravenously to maintain a plasma concentration of approximately 20 mg/100 ml.

The concentration of inulin in the plasma or urine samples was estimated by spectrophotometry (Heyrousky, 1956) using a continuous flow auto-analyser system (Dawborn, 1965). In brief, the plasma or urine samples were diluted with 0.5N hydrochloric acid and the inulin hydrolysed to fructose by heating at 60°C for 10 min. Protein in the samples was removed by dialysis and colour developed by treating the protein-free dialysate with concentrated hydrochloric acid and 3-indoleacetic acid at 60°C for 8 min. The colour produced in the samples was estimated in a colorimeter at 520 nm, recorded on a chart recorder, and compared with the colour produced by standard solutions.

Glomerular filtration rate, expressed as ml/min, was taken to be inulin clearance (Cinulin) and was calculated by:

$$Cinulin = \frac{Uinulin}{Pinulin} \times \dot{V}$$

where Uinulin = concentration of inulin in urine (mg %)
Pinulin = concentration of inulin in plasma (mg %)
$\dot{V}$ = urine flow (ml/min)

From PAH and inulin clearances, filtration fraction can be calculated:

$$\text{Filtration fraction} = \frac{Cinulin}{C_{PAH}}$$

2.5.3 *Estimation of urinary and plasma sodium and potassium concentration*

Urinary and plasma sodium and potassium concentrations were estimated by flame photometry (Corning 435 flame photometer). The flame photometer was calibrated using Corning prepared standard solutions of 200 mM/litre sodium and 100 mM/litre potassium using 3M

lithium as internal standard. The sodium and potassium signals were ratioed with the lithium reference signal to prevent changes in signal level caused by fluctuations in the flame.

Sodium excretion ($U_{Na^+}V$) and potassium excretion ($U_{K^+}V$), expressed as μM/min, were calculated by:

$$U_{Na^+}V = U_{Na^+} \times \dot{V}$$

where U_{Na^+} = concentration of sodium in the urine (μM/ml)

$\dot{V}$ = urine flow (ml/min)

$$U_{K^+}V = U_{K^+} \times \dot{V}$$

where U_{K^+} = concentration of potassium in the urine (μM/ml)

$\dot{V}$ = urine flow (ml/min)

2.5.4 Estimation of urinary and plasma osmolality

Urinary and plasma osmolality was estimated by freezing point determination on the principle that the colligative properties of a solution change in proportion to the number of solute particles present. The osmometer (Advanced Instruments, Digimatic Osmometer, 3D11) was calibrated using a series of prepared standards, and the osmolality of the sample expressed as m osmoles/kg of water.

Solute excretion ($U_{OSM}V$), expressed as μ osm/min, was calculated by:

$$U_{OSM}V = U_{OSM} \times \dot{V}$$

where U_{OSM} = concentration of osmoles in urine (m osm/kg H_2O)

$\dot{V}$ = urine flow (ml/min)

Osmolality clearance (C_{OSM}), expressed as ml/min, was calculated by:

$$C_{OSM} = \frac{U_{OSM}}{P_{OSM}} \times \dot{V}$$

where U_{OSM} = osmolality of urine (m osmol/kg H_2O)
P_{OSM} = osmolality of plasma (m osmol/kg H_2O)
$\dot{V}$ = urine flow (ml/min)

Free water clearance, expressed as ml/min, can be calculated by:

$$C_{H_2O} = \dot{V} - C_{OSM}$$

2.5.5 Estimation of plasma renin activity

Plasma renin activity was estimated using radioimmunoassay to measure the quantity of angiotensin 1 generated by plasma samples under standardised conditions. A commercially available radioimmunoassay kit was used (CIS Cea Sorin angiotensin 1 radioimmunoassay kit, USA).

In brief, an aliquot of a plasma sample was incubated at 37°C, pH 5.5-6.0 for 1.5 h to maximise the action of renin. 2,3-dimercaptopropanol and 8-hydroxyquinoline were added to prevent degradation of the angiotensin 1. Another aliquot of the plasma sample was incubated at 2-4°C as a sample blank. After incubation, the reaction was stopped by lowering the temperature to 2-4°C. Radioimmunoassay was then performed upon the generated angiotensin 1, and the angiotensin 1 standards. Angiotensin 1 competed with the radioactive tracer for the binding sites of the antibody and the immune complex formed was separated from the free angiotensin 1 by adsorbing the latter on dextran-coated charcoal. After centrifugation of the charcoal, the supernatant

was decanted into scintillation vials and the bound radioactivity counted in a γ-counter. Finally, a standard curve was plotted and the angiotensin 1 concentration in the plasma sample determined.

The plasma renin activity (PRA), expressed as ng angiotensin 1/ml/hr, was calculated by:

$$PRA = \frac{a - b}{t}$$

where a = angiotensin 1 concentration in a sample aliquot incubated at 37°C (ng/ml)

b = angiotensin 1 concentration in a sample aliquot kept at low temperature (ng/ml)

t = time of incubation at 37°C (hr)

The intra-assay coefficient of variation was approximately 6% while the inter-assay coefficient of variation was approximately 10%.

2.6 Estimation of Urinary 6-keto-$PGF_{1\alpha}$ by Stable Isotope Dilution and Combined Gas Chromatography-Mass Spectrometry

Before the quantification of urinary 6-keto-$PGF_{1\alpha}$ by GC-MS was performed, the prostaglandin was extracted from the urine and the extract purified in order to secure a high specificity of the assay and optimise the signal : noise ratio.

2.6.1 Extraction and purification of 6-keto-$PGF_{1\alpha}$ from urine

(a) Amberlite XAD-2 extraction

6-keto-$PGF_{1\alpha}$ was extracted from up to 50 ml volumes of urine. To each urine sample, a constant amount of 3,3,4,4-tetradeuterated 6-keto-$PGF_{1\alpha}$ (100 ng in 20 µl MeOH) (d_4 6-keto-$PGF_{1\alpha}$, a gift from the Upjohn Company, USA) and 5,8,9,11,12,14,15-tritiated 6-keto-$PGF_{1\alpha}$ (0.025 µCi in 50 µl MeOH) (^{3}H-6-keto-$PGF_{1\alpha}$, Amersham Radiochemicals,

UK) was added. A sufficient quantity of amberlite XAD-2 (BDH Chemicals Ltd, UK) was washed thoroughly in methanol, acetone and distilled water, fine particles and colloidal material being decanted. After the washing procedure, the XAD-2 was suspended in distilled water and the columns packed by sedimentation using 3 gm XAD-2/column, held in place by a plug of glass wool. Urine samples were acidified to pH 3.0 - 3.5 with 1N hydrochloric acid and percolated through the columns. The columns were washed with 2 x 10 ml distilled water before the 6-keto-$PGF_{1\alpha}$ was eluted with 2 x 10 ml methanol. The eluate was taken to dryness on a rotary evaporater at 45°C.

(b) Silicic acid chromatography

In this chromatographic step, three solvent systems were used:

System I: toluene : ethyl acetate (60 : 40)
System II: toluene : ethyl acetate : methanol (60 : 40 : 0.5)
System III: toluene : ethyl acetate : methanol (60 : 40 : 20)

Columns were packed with an equilibrated slurry of activated silicic acid (Unisil 100-200 mesh, Clarkson Chemical Co., USA) in solvent system I, using 1.5 gm silicic acid/column held in place by a plug of glass wool. The columns were washed with 6 ml solvent system III followed by 6 ml solvent system I. The urinary extracts were then applied to the columns dissolved in a mixture of 4 ml solvent system I and 1 ml solvent system III. The columns were washed with 8 ml solvent system II before the 6-keto-$PGF_{1\alpha}$ was eluted with 8 ml solvent system III. The eluate was evaporated under air.

(c) High pressure liquid chromatography

Straight phase high pressure liquid chromatography (HPLC) was performed using a partsil PAC column of 10 cm length and 0.4 cm internal diameter (Whatman Inc., USA). The column was fitted with a 1 ml loop injector (Rheodyne, USA) and connected to a Perkin Elmer Series 2 liquid chromatograph. A pre-column packed with Co : Pell PAC (HPLC Technology Ltd, UK) was added to the system to remove any particulate material from the sample and hence prolong the chromatographic efficiency of the main column. A solvent system of dichloroethane : methanol : acetic acid (90 : 10 : 0.1) was used, and after equilibration of the column, the flow rate was set at 1 ml/min. Samples were injected dissolved in 300 µl of the solvent system and 1 ml fractions of the column eluate were collected using an Ultorac fraction collector (LKB Ltd, Sweden).

As 6-keto-$PGF_{1\alpha}$ contains no chromophore, it was not possible to monitor its elution time by ultra-violet absorption. The tritiated 6-keto-$PGF_{1\alpha}$ added to each urine sample before extraction was therefore used as a marker. 100 µl aliquots of the 1 ml fractions of the column eluate were added to 10 ml scintillation fluid (10 gm PPO and 0.5 gm dimethyl POPOP dissolved in 2.5 l toluene) and counted in a liquid scintillation counter (Nuclear Chicago Ltd, UK). The relevant fractions were combined and taken to dryness under air.

During the purification of a number of samples by HPLC, it was necessary to ensure that the chromatographic properties of the column remained stable. PGB_2 has similar chromatographic properties to 6-keto-$PGF_{1\alpha}$ but is distinct from other prostaglandins in that it contains a chromophore and therefore absorbs ultra-violet light. The chromatographic properties of the column were checked by repeatedly noting the

elution time of PGB_2 by monitoring the ultraviolet absorption using a Cecil CE212 variable wavelength spectrophotometer (Cecil Instruments, UK). The absorption wavelength was set at 280 nm.

2.6.2 *Recovery of 6-keto-PGF$_{1\alpha}$ from extraction and purification Procedures*

Although the use of d_4 6-keto-$PGF_{1\alpha}$ as internal standard accounted for the loss of material during the extraction and purification procedures, calculation of recoveries of 6-keto-$PGF_{1\alpha}$ proved useful in indicating the overall variability of the method. Mean recoveries of 40% were found after the three successive chromatographic stages, the bulk of the material being lost at the silicic acid stage.

2.6.3 *Derivatisation of 6-keto-PGF$_{1\alpha}$*

Prior to GC-MS, the 6-keto-$PGF_{1\alpha}$ had to be derivatised to a molecule with the most suitable characteristics for mass spectrometry, whilst at the same time possessing satisfactory gas chromatographic properties. The methyl ester, methoxime, trimethyl silyl; methyl ester, ethoxime, trimethyl silyl; and methyl ester, butoxime, trimethyl silyl ether derivatives of 6-keto-$PGF_{1\alpha}$ were prepared and their mass spectra compared. The methyl ester, methoxime, trimethyl silyl ether derivative (6-keto-$PGF_{1\alpha}$ Me, MO, TMS) was found to be the most suitable since it demonstrated ions in its mass spectrum of high relative abundance and high mass, conferring sensitivity and specificity to the assay (Figure 2.2).

The methyl ester was prepared by treatment of a methanolic solution of the sample with an excess of an ethereal solution of diazomethane. The methanol in the sample has been found to be necessary for catalysing the reaction (Schlenk and Gellerman, 1960). Diazomethane

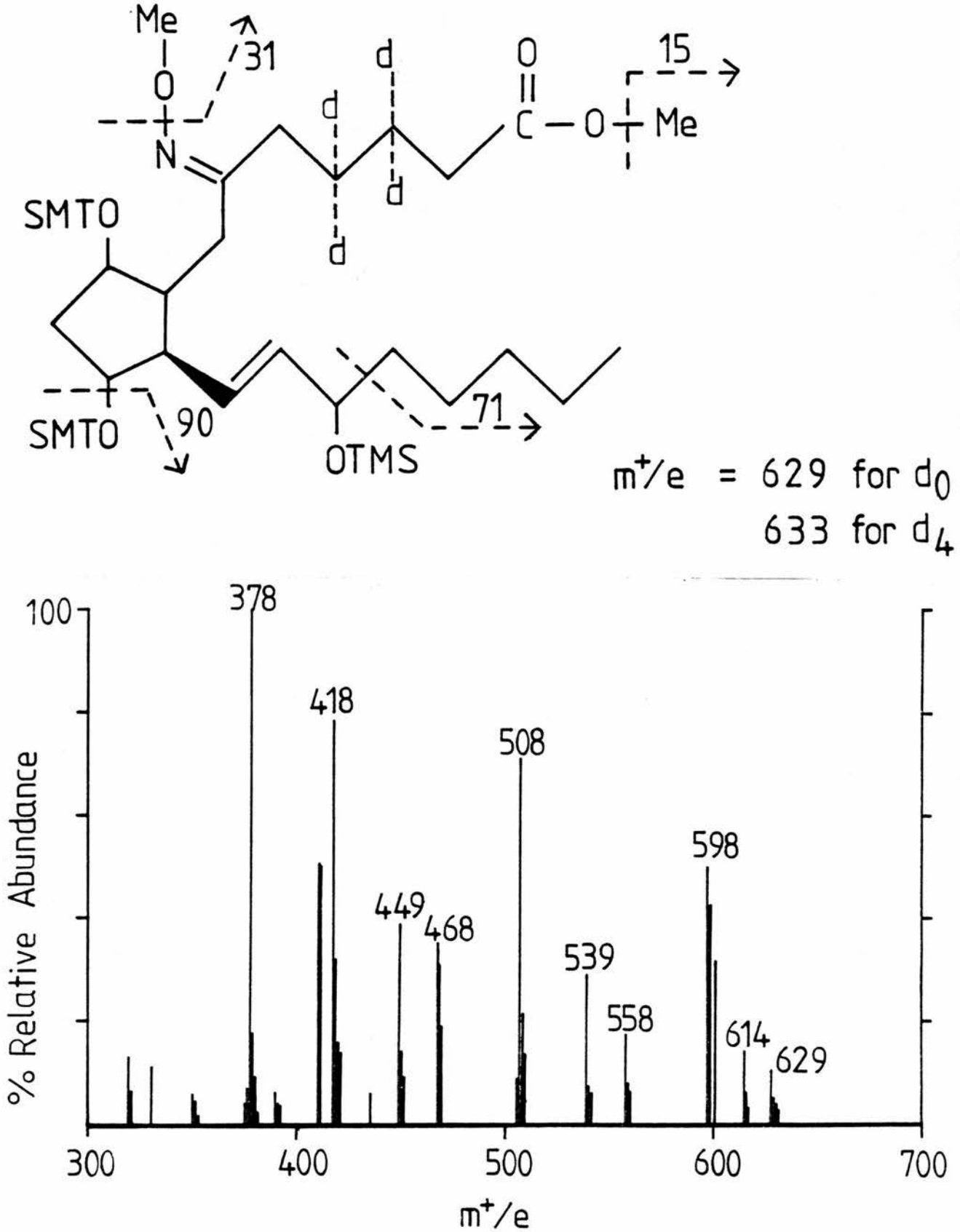


FIGURE 2.2: The methyl ester, methoxime, trimethyl silyl derivative of 6-keto-$PGF_{1\alpha}$ and its mass spectrum.

was prepared by the reaction of ethereal N-methyl-N-nitroso-p-toluene-suphonamide (Aldrich Chemicals Co., UK) with aqueous potassium hydroxide and ethanol, and transferred into fresh diethyl ether with the aid of a stream of nitrogen. This ethereal solution could be satisfactorily stored at -20°C for short periods. The sample was allowed to stand in the diazomethane for five minutes at room temperature before being blown to dryness and vacuum dessicated.

The methyl oxime was prepared by treatment of the esterified material with 100 µl of a 5 mg/ml solution of methoxyamine hydrochloride (Supelco Inc., USA) in pyridine. The reaction was left to proceed overnight at room temperature, then the samples were treated at 60°C for 40 min before being blown to dryness and vacuum dessicated.

The methyl ester, methoxime derivative was then reacted to form the trimethyl silyl ether by dissolving the esterified, oximated material in 20 µl bis (trimethyl silyl) tri-fluoro-acetamide containing 1% trimethyl-chlorosilane (Sigma Chemical Co., USA). The reaction was carried out at 60°C for 20 min.

2.6.4 Quantification of 6-keto-PGF$_{1\alpha}$ by gas chromatography-mass spectrometry

The estimations of 6-keto-PGF$_{1\alpha}$ in the urinary extracts were obtained using a VG-Micromass 70-70F mass spectrometer coupled to a PYE 204 gas chromatograph by means of a single stage glass separator. The gas chromatograph was equipped with a spiral glass column (1.25 m x 4 mm i.d.) packed with 3% OV-1 on Supelcoport 100-200 mesh (Supelco Inc., USA). The carrier gas in the gas chromatograph was helium and the flow rate of the helium and the temperature of the column were adjusted to obtain a suitable retention time for the 6-keto-PGF$_{1\alpha}$ Me, MO, TMS. The retention time should be long enough to achieve good

chromatographic separation but short enough to render the assay practical. The column temperature and helium flow rate were set to give the 6-keto-$PGF_1\alpha$ Me, MO, TMS a retention time of approximately 7.5 min (approximately 250°C; 2.0 ml/min).

The chromatographic properties of the column tended to vary from day to day even at a given temperature and helium flow rate. This variability was dependent upon the extent to which the GC-MS was being used, such that extensive use of the gas chromatograph resulted in longer retention times. It was therefore desirable to obtain an indication of the chromatographic efficiency of the column prior to commencing an assay. This was achieved by plotting the retention times of a mixture of fatty acid methyl esters on a logarithmic scale against the number of carbon atoms in the esterified carboxylic acids (Bergström, Ryhage, Samuelsson and Sjovall, 1963). This graph was used to convert the observed retention time of the 6-keto-$PGF_1\alpha$ Me, MO, TMS into a carbon value. Having determined the carbon value, the graph could then be used to indicate the expected retention time of the 6-keto-$PGF_1\alpha$ Me, MO, TMS prior to each assay allowing any necessary adjustments in the column temperature to be made. 6-keto-$PGF_1\alpha$ Me, MO, TMS had a carbon value of 25.

Typical mass spectrometer operating conditions were:

Separator temperature:	250°C
Ion source temperature:	250°C
Electron energy:	22-70 eV (dependent on instrument performance)
Accelerating voltage:	4 kV

For quantitative determinations, the mass spectrometer was used in the multiple ion detection mode with fixed magnet current and voltage scanning. The mass spectrometer was also operated at high resolution

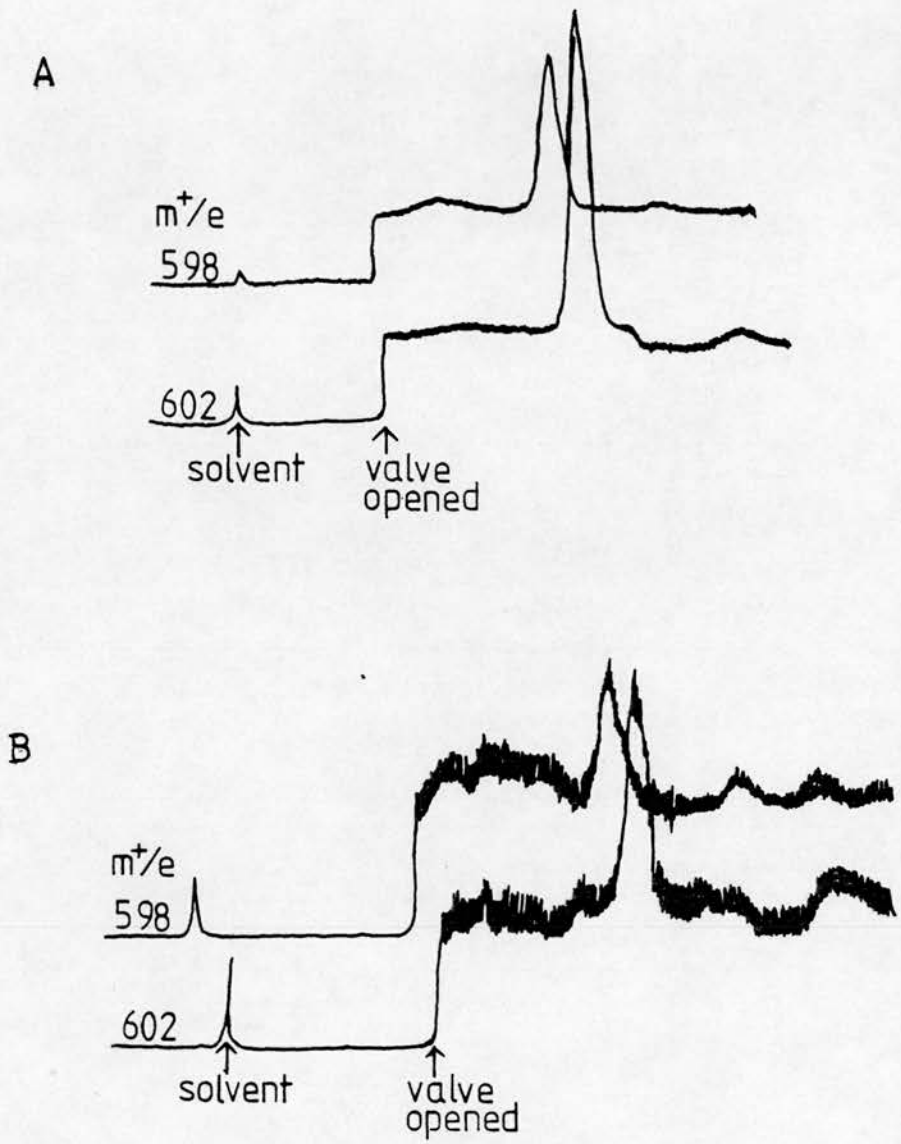


FIGURE 2.3: Representative ion chromatograms of Me, MO, TMS, 6-keto-$PGF_{1\alpha}$.

A - ion chromatogram of 6-keto-$PGF_{1\alpha}$ standard (machine gain 5V).

B - ion chromatogram of 6-keto-$PGF_{1\alpha}$ from urine sample (machine gain 50V).

enabling separation of ions with very close masses. Although this resulted in some loss in sensitivity, it ensured a high specificity of the assay.

Heptacosafluorotributylamine (V.G. Micromass, UK) was bled slowly into the mass spectrometer to produce a series of ion fragments of known exact masses. The mass spectrometer was then tuned to the selected 6-keto-$PGF_{1\alpha}$ Me, MO, TMS ion fragment by reference of its precise mass to the precise mass of the nearest heptacosafluorotributylamine ion fragment.

For the Me, MO, TMS derivative of 6-keto-$PGF_{1\alpha}$, the mass spectrometer was focussed to monitor the ion intensity at m/e 598 and the corresponding deuterated ion, m/e 602, using a reference heptacosafluorotributylamine ion intensity of 501. Although the 598 ion of the 6-keto-$PGF_{1\alpha}$ Me, MO, TMS did not have such a large relative abundance as the 418 or 508 ions (Figure 2.2), it was found necessary to use the ion of higher mass in order to avoid interference from contaminants in the sample.

Standards were composed of known quantities of derivatised unlabelled and deuterated 6-keto-$PGF_{1\alpha}$, the amount of deuterated material being the same as that added to the urine samples prior to extraction. Aliquots (5 µl) of the standards and urinary extracts were injected into the GC-MS and the ion currents at the m/e 598 and 602 recorded simultaneously as single ion chromatograms on a multi-pen recorder (Rika denki Mitsui, Electronics Ltd, UK). Representative chromatograms obtained from samples of dog urine are shown in Figure 2.3. Calibration curves were constructed by plotting the quantity of unlabelled 6-keto-$PGF_{1\alpha}$ in each standard against the peak height ratio m/e 598/602 (Figure 2.4). The peak height ratios m/e 598/602 for the urinary extracts were

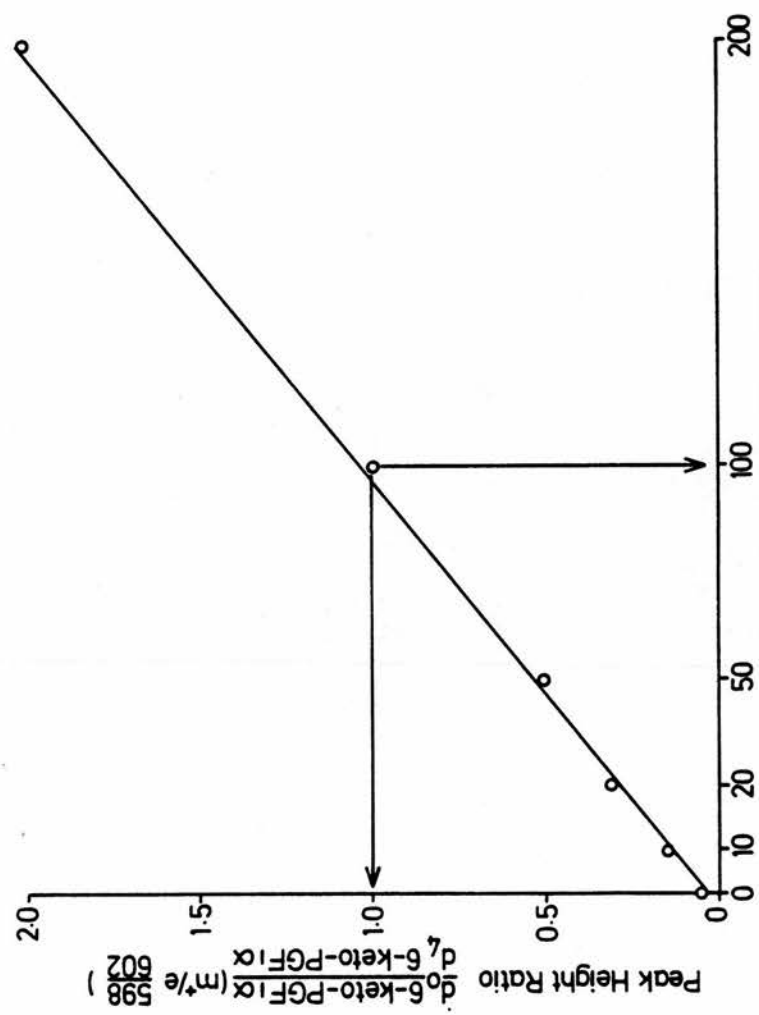


FIGURE 2.4: Standard curve for 6-keto-$PGF_{1\alpha}$ as performed by GC-MS.

calculated and the urinary 6-keto-$PGF_{1\alpha}$ level estimated from the standard curve.

A standard curve was constructed for each assay performed. Any minor variation seen between standard curves was most likely due to inaccuracy in standard preparation.

The y-intercept (0.05) of the standard curve was due, primarily, to the inevitable presence of unlabelled molecules in the deuterated 6-keto-$PGF_{1\alpha}$ and the value 0.05 sets the lower limit of sensitivity of the method with a minimum measurable ratio set arbitrarily at 2 x 0.05. Using 100 ng d_4 6-keto-$PGF_{1\alpha}$ added to 50 ml dog urine, the normal endogenous levels of 6-keto-$PGF_{1\alpha}$ (0.4 - 2.6 ng/ml) gave m/e 598/602 ratios in the range 0.3 - 1.2. A 6-keto-$PGF_{1\alpha}$ excretion rate of less than 35% of normal should therefore be measurable.

2.6.5 Parameters of the analytical method

(a) Precision

In order to determine how precisely the GC-MS technique could quantify a known amount of 6-keto-$PGF_{1\alpha}$ from a urine sample, and to ensure that this precision was not affected by the initial volume of the sample, the following test was performed.

In addition to the internal standard, 200 ng unlabelled 6-keto-$PGF_{1\alpha}$ was added to each of six aliquots of the same urine collection (10 ml, 25 ml, 50 ml, 100 ml, 150 ml, 200 ml). A seventh aliquot of 50 ml was extracted without prior 6-keto-$PGF_{1\alpha}$ addition. Following purification and derivatisation, the 6-keto-$PGF_{1\alpha}$ in the urine was quantified by GC-MS. The results in Table 2.1 represent the estimated amounts of 6-keto-$PGF_{1\alpha}$ in the various urinary aliquots following subtraction of the endogenous level. The mean value for the 6-keto-$PGF_{1\alpha}$ quantified from the urine samples represents 93% of the original material.

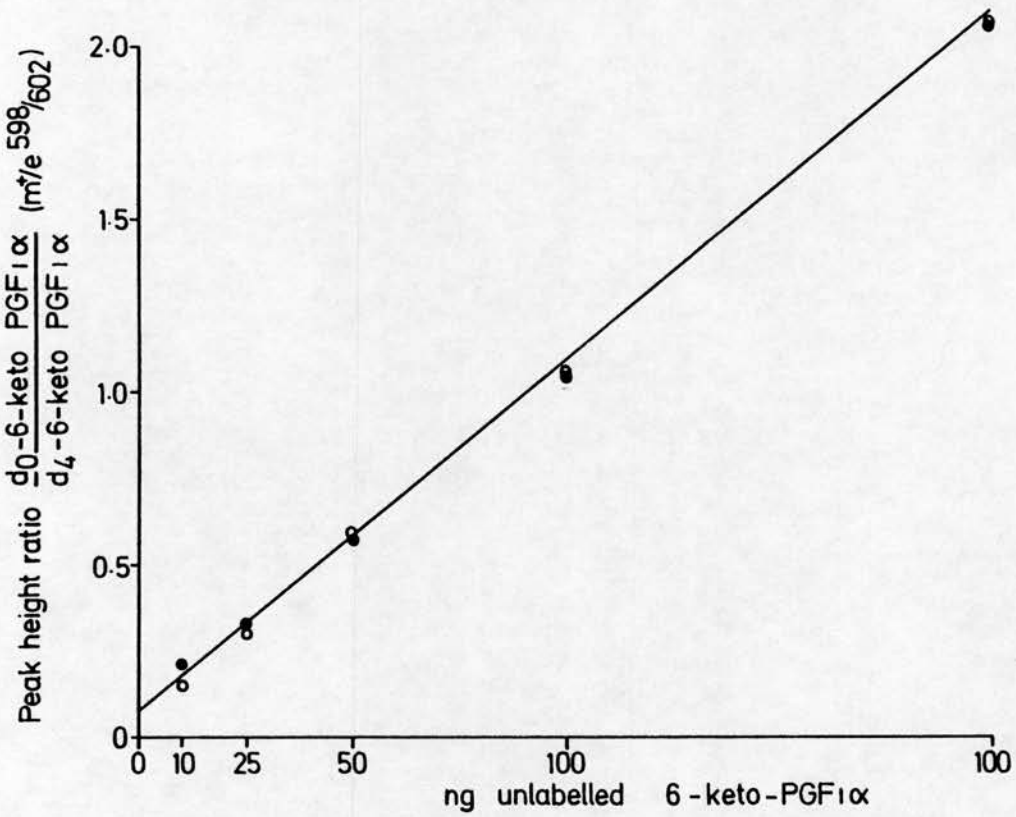


FIGURE 2.5: Comparison of 6-keto-$PGF_{1\alpha}$ standard curves using standards which have been extracted from urine prior to derivatisation and standards which have been derivatised only.

- • represents unextracted standards;
- o represents extracted standards.

TABLE 2.1:

Volume of urine sample (ml)	10	25	50	100	150	200
Quantity of 6-keto-$PGF_{1\alpha}$ (ng)	197	197	180	175	195	177

(b) Accuracy

In order to ensure that the accuracy of the method was not affected by the prior chromatographic procedures, the following test was performed.

Two standard curves were constructed, one set of standards having been added to 50 ml aliquots of the same urine collection and taken through the extraction procedure prior to derivatisation. After accounting for the endogenous 6-keto-$PGF_{1\alpha}$ in the urine, there was good agreement between the ratios obtained for the standards taken through the extraction procedure and those derivatised without prior extraction (Figure 2.5). Standards derivatised without prior extraction were therefore routinely employed for the calibration curves.

(c) Sensitivity

The lower limit of measurement varied with two parameters. Firstly, the signal/noise ratio of the particular urine extract and, secondly, the sensitivity of the mass spectrometer. The sensitivity of the mass spectrometer fluctuated markedly from day to day reflecting variation in the condition of the ion source.

Generally, a 6-keto-$PGF_{1\alpha}$ concentration of 0.2 ng/ml could be measured from a urine sample.

SECTION III

Determination of the Physiological Significance of Systemic PGI_2 and Its Relationship to Urinary 6-keto-$PGF_{1\alpha}$

3.1 Introduction

PGI_2 infused intravenously into man (Szczeklik, Szczeklik and Nizankowski, 1980) or anaesthetised animals (Armstrong, Dusting, Moncada and Vane, 1978) lowered systemic blood pressure by decreasing total peripheral vascular resistance. Infusion of large doses of PGI_2 (>30 ng/kg/min) into the renal artery of anaesthetised dogs increased renal blood flow and also resulted in a decrease in systemic blood pressure (Gerber *et al.*, 1978b). This indicated that PGI_2 in the renal venous blood was recirculated to cause a decrease in total peripheral vascular resistance and raised the possibility that PGI_2 may have a role as a circulating as well as a local hormone. The demonstration that PGI_2 was present in both arterial and venous blood (Moncada, Korbut, Bunting and Vane, 1978) and was released from the lung into the arterial circulation (Gryglewski, Korbut and Ocetkiewicz, 1978) led to the hypothesis that PGI_2 may act as a circulating hormone in the control of systemic blood pressure.

If PGI_2 were to be acting as a circulating hormone, it is possible that systemic PGI_2 may exert control on renal haemodynamics and electrolyte excretion. It would therefore be important to investigate the effects of intravenously infused PGI_2 on these parameters.

Hill and Moncada (1979) infused PGI_2 intravenously into anaesthetised dogs at a rate of 300 ng/kg/min. Although there was little change in renal blood flow, the significant fall in systemic blood pressure caused by the high concentration of PGI_2, resulted in decreases in glomerular filtration rate, urine volume and electrolyte excretion (Hill and Moncada, 1979). In this and other studies investigating the haemodynamic actions of PGI_2, anaesthetised, surgically traumatised animal preparations have been used. The use of conscious

surgically untraumatised animals would be more valuable for the evaluation of the physiological role of PGI_2, particularly its role in the control of renal haemodynamics and electrolyte excretion.

In the absence of specific antagonists or synthesis inhibitors, useful information about the physiological actions of PGI_2 can be obtained from two means of investigation. First, by observing the effects of infusion of low concentrations of PGI_2 in unanaesthetised animals and, secondly, by measuring its rate of production under normal and stimulated conditions.

6-keto-$PGF_{1\alpha}$ can be detected in the urine but it is not possible to determine whether this reflects circulating and/or renal production of PGI_2 since in addition to systemic vascular synthesis, many sites within the kidney are capable of synthesising PGI_2 (Grenier and Smith, 1978; Terragno *et al.*, 1978; Hassid *et al.*, 1979). Tracer studies have demonstrated that, as well as being hydrolysed to 6-keto-$PGF_{1\alpha}$, PGI_2 is enzymatically metabolised by the blood vessels (Wong *et al.*, 1978), kidney (Wong *et al.*, 1979) and liver (Wong *et al.*, 1980). Like 6-keto-$PGF_{1\alpha}$, these enzymatically produced metabolites are excreted in the urine (Sun and Taylor, 1978). Assuming that endogenous PGI_2 is metabolised in the same way as exogenous PGI_2 and that the metabolites are excreted in a similar manner, it is possible that endogenous PGI_2 production may be more reliably reflected by measurement of one or more of its enzymatically produced metabolites rather than by measurement of urinary 6-keto-$PGF_{1\alpha}$. At the time of this study, although the structures of the major urinary metabolites of PGI_2 had been elucidated, standards to allow their measurement were not available. It was therefore pertinent to determine the precise relationship between systemic PGI_2 and urinary excretion of 6-keto-$PCF_{1\alpha}$.

The aim of this section was to study the effects of intravenously infused PGI_2 on systemic and renal haemodynamics and electrolyte excretion and to relate this to the urinary excretion of 6-keto-$PGF_{1\alpha}$, thereby allowing assessment of the physiological significance of PGI_2.

3.2 Methods

Eight male conscious foxhounds were used, all animals being surgically prepared as described in Section 2.1. On the day of each study, sterile dextrose solution (5 gm/100 ml) was infused at 7 ml/min for 30 min, then continued at 3 ml/min throughout the rest of the 4 hr study period. This established a modest water diuresis. Loading doses of PAH and inulin were administered, and the appropriate dilutions intravenously infused at 0.5 ml/min. A one-hour equilibration period was allowed. Vehicle buffer or PGI_2, in concentrations of 7.5, 15 or 30 ng/kg/min, was infused intravenously at 0.5 ml/min for 4 hr. The PGI_2 was dissolved in 0.025M carbonate buffer at pH 9.8. In this buffer, PGI_2 has a half-life of 13 hr at room temperature (Cho and Allen, 1978). This was confirmed by detecting by spectrophotometry, the appearance of 6-keto-$PGF_{1\alpha}$ in a solution of PGI_2. In order to minimise the quantity of hydrolysed PGI_2 infused, the infusion solution was replaced every 30 min by a fresh solution prepared from a PGI_2 stock kept on ice. During several studies, the activity of the PGI_2 was checked at intervals throughout the 4 hr period. This was achieved by comparing the ability of the PGI_2 in the infusion solution to prevent ADP-induced platelet aggregation, with that of freshly prepared standards. In all cases, the PGI_2 in the infusion solution had the predicted activity.

Throughout the 4 hr infusion periods, systemic blood pressure was monitored continuously, urine collections were made every 30 min and arterial blood samples taken every 60 min (see Section 2.4.3). Renal plasma flow, glomerular filtration rate, sodium, potassium and osmolality excretion, plasma renin activity and urinary 6-keto-$PGF_{1\alpha}$ were estimated by techniques described fully in Section 2.5.

At least 48 hr were allowed between each study.

3.3 Statistical Comparisons

The object of the study was to compare the effect of buffer infusion with infusion of different concentrations of PGI_2, over time. This protocol is typical of a repeat measurements study and necessitates the use of the analysis of variance as a statistical method. The analysis of variance assumes that the measurements were obtained under independent conditions, that the data were normally distributed and that each group had the same underlying standard deviation. In the repeated measurements analysis of variance, the total variability is partitioned into differences between experimental units, variation over time, and residual variability. The analysis of variance therefore comprises of three F-statistics performed using a critical F value according to Greenhouse and Geisser ($F_{0.05} = 1(n-1)$).

The first F-statistic (F_1) examines the variability between time points between drugs. If this test is not significant then the trend over time is similar for each treatment.

The second F-statistic (F_2) looks at the trend over time. If this test is not significant then there is no trend over time, that is, the variable remains constant throughout the study.

The third F-statistic (F_3) examines the difference between treatments with an underlying test for variability between animals. This is performed by comparing means over total time and therefore to be valid, it requires that the first F-test is not significant, i.e. all treatments must show the same trend.

Significance for all F-tests was taken at $p < 0.05$.

The large daily fluctuations in plasma renin activity necessitated a control blood sample for estimation of plasma renin activity to be taken immediately prior to each study. The effect of PGI_2 on plasma renin activity was therefore statistically compared with the control value using a student 2 tailed t-test. Significance was taken at $p < 0.05$.

Although most statistical comparisons have been made using analysis of variance, for convenience, the data in the tables and graphs in the following sub-section are expressed as mean ± standard error.

3.4 Results

All data are summarised in Tables 3.1 - 3.3, and the values for statistical analyses are summarised in Table 3.4.

3.4.1 Systemic blood pressure (Fig. 3.1)

Statistical analysis demonstrated a similar, significant trend in systemic blood pressure in all four infusions (F_1, $p < 0.1257$; F_2, $p < 0.0001$). This trend was for systemic blood pressure to increase during each study, as observed by the small progressive increase during the vehicle buffer and 7.5 ng/kg/min PGI_2 infusion and the more pronounced increases following the initial depression in systemic blood pressure observed during the 15 and 30 ng/kg/min PGI_2 infusions.

TABLE 3.1

Parameter	Time (minutes) 30	60	90	120	150	180	210	240
Systemic Blood Pressure (mm Hg)								
Buffer	96.8 ± 1.8	96.3 ± 1.6	97.3 ± 2.6	99.0 ± 3.0	101.0 ± 2.1	102.0 ± 2.8	100.7 ± 2.6	102.0 ± 2.2
PGI_2 7.5 ng/kg/min	93.8 ± 2.6	97.0 ± 2.4	97.3 ± 2.1	94.7 ± 2.6	97.4 ± 2.6	101.8 ± 2.2	101.8 ± 2.2	102.1 ± 2.7
PGI_2 15 ng/kg/min	90.4 ± 3.6	87.0 ± 3.6	93.8 ± 1.6	92.7 ± 3.3	95.0 ± 3.4	95.7 ± 3.9	97.4 ± 2.7	98.8 ± 2.1
PGI_2 30 ng/kg/min	87.8 ± 2.8	86.0 ± 1.6	92.1 ± 1.9	94.0 ± 2.4	95.2 ± 3.3	96.7 ± 3.4	96.8 ± 3.0	97.2 ± 2.9
Heart Rate (beats/min)								
Buffer	86 ± 6	89 ± 4	82 ± 8	86 ± 6	91 ± 10	87 ± 7	91 ± 5	91 ± 5
PGI_2 7.5 ng/kg/min	84 ± 5	79 ± 7	82 ± 6	98 ± 10	84 ± 5	96 ± 7	81 ± 4	83 ± 4
PGI_2 15 ng/kg/min	88 ± 11	88 ± 8	92 ± 7	85 ± 6	84 ± 6	86 ± 4	91 ± 5	88 ± 6
PGI_2 30 ng/kg/min	84 ± 6	90 ± 8	77 ± 8	90 ± 7	83 ± 7	91 ± 8	87 ± 8	88 ± 5
Renal Plasma Flow (ml/min)								
Buffer	337 ± 48	263 ± 45	307 ± 27	304 ± 46	305 ± 48	280 ± 36	282 ± 41	312 ± 51
PGI_2 7.5 ng/kg/min	375 ± 90	313 ± 54	376 ± 94	349 ± 46	354 ± 47	385 ± 50	352 ± 37	314 ± 42
PGI_2 15 ng/kg/min	311 ± 43	302 ± 40	299 ± 39	308 ± 43	356 ± 38	283 ± 34	323 ± 23	349 ± 46
PGI_2 30 ng/kg/min	332 ± 63	301 ± 48	426 ± 72	352 ± 56	363 ± 48	347 ± 56	353 ± 46	360 ± 38
Glomerular Filtration Rate (ml/min)								
Buffer	93 ± 7	83 ± 11	92 ± 7	84 ± 7	91 ± 10	82 ± 6	83 ± 7	95 ± 7
PGI_2 7.5 ng/kg/min	102 ± 17	87 ± 7	96 ± 14	94 ± 5	81 ± 14	94 ± 11	98 ± 8	88 ± 8
PGI_2 15 ng/kg/min	92 ± 9	88 ± 9	99 ± 15	89 ± 12	94 ± 7	90 ± 12	90 ± 7	91 ± 9
PGI_2 30 ng/kg/min	92 ± 11	71 ± 4	101 ± 8	92 ± 8	87 ± 11	85 ± 12	91 ± 12	96 ± 9
Filtration Fraction								
Buffer	0.27 ± 0.02	0.31 ± 0.15	0.29 ± 0.03	0.27 ± 0.03	0.30 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.30 ± 0.03
PGI_2 7.5 ng/kg/min	0.27 ± 0.02	0.27 ± 0.03	0.25 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	0.24 ± 0.02	0.28 ± 0.02	0.28 ± 0.03
PGI_2 15 ng/kg/min	0.29 ± 0.02	0.29 ± 0.03	0.33 ± 0.04	0.28 ± 0.02	0.26 ± 0.02	0.31 ± 0.02	0.27 ± 0.02	0.26 ± 0.02
PGI_2 30 ng/kg/min	0.27 ± 0.02	0.23 ± 0.02	0.24 ± 0.03	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.25 ± 0.02	0.26 ± 0.02

TABLE 3.2

Parameter	Time (minutes) 30	60	90	120	150	180	210	250
Sodium Excretion (μM/min)								
Buffer	60 ± 25	57 ± 32	52 ± 27	43 ± 13	48 ± 18	59 ± 19	61 ± 14	70 ± 21
PGI_2 7.5 ng/kg/min	60 ± 29	50 ± 34	48 ± 21	81 ± 19	85 ± 26	101 ± 25	118 ± 22	94 ± 18
PGI_2 15 ng/kg/min	41 ± 15	34 ± 17	48 ± 18	49 ± 17	64 ± 20	67 ± 15	91 ± 21	94 ± 17
PGI_2 30 ng/kg/min	46 ± 13	52 ± 14	56 ± 13	43 ± 9	63 ± 10	64 ± 18	76 ± 17	93 ± 18
Potassium excretion (μM/min)								
Buffer	38 ± 6	32 ± 6	37 ± 6	36 ± 5	40 ± 7	44 ± 7	37 ± 6	44 ± 7
PGI_2 7.5 ng/kg/min	49 ± 9	36 ± 6	52 ± 12	56 ± 7	46 ± 5	52 ± 9	53 ± 6	49 ± 9
PGI_2 15 ng/kg/min	36 ± 5	28 ± 6	40 ± 8	39 ± 6	42 ± 5	41 ± 6	39 ± 6	40 ± 7
PGI_2 30 ng/kg/min	42 ± 7	41 ± 4	56 ± 9	51 ± 12	53 ± 7	47 ± 9	48 ± 7	52 ± 6
Urine Flow (ml/min)								
Buffer	4.4 ± 0.5	3.7 ± 0.5	3.9 ± 0.3	4.1 ± 0.4	3.8 ± 0.4	3.0 ± 0.4	2.9 ± 0.3	3.5 ± 0.4
PGI_2 7.5 ng/kg/min	4.1 ± 0.6	3.4 ± 0.3	3.4 ± 0.5	3.5 ± 0.5	3.4 ± 0.6	3.7 ± 0.5	3.8 ± 0.4	3.4 ± 0.3
PGI_2 15 ng/kg/min	3.3 ± 0.4	2.3 ± 0.6	3.3 ± 0.5	3.2 ± 0.3	3.9 ± 0.6	3.0 ± 0.4	2.9 ± 0.2	3.7 ± 0.7
PGI_2 30 ng/kg/min	3.6 ± 0.6	2.4 ± 0.5	3.9 ± 0.4	3.7 ± 0.7	4.1 ± 0.6	3.4 ± 0.2	3.5 ± 0.5	4.0 ± 0.4
Osmolality Excretion (μOsm/min)								
Buffer	644 ± 86	542 ± 92	594 ± 74	492 ± 52	537 ± 69	527 ± 63	486 ± 45	567 ± 54
PGI_2 7.5 ng/kg/min	645 ± 66	538 ± 48	604 ± 58	661 ± 63	560 ± 87	631 ± 58	695 ± 64	580 ± 49
PGI_2 15 ng/kg/min	533 ± 40	476 ± 60	496 ± 31	487 ± 52	545 ± 37	469 ± 40	530 ± 39	538 ± 63
PGI_2 30 ng/kg/min	524 ± 45	538 ± 82	653 ± 69	508 ± 63	539 ± 65	527 ± 75	464 ± 58	650 ± 86
Free Water Clearance (ml/min)								
Buffer	2.2 ± 0.3	2.7 ± 0.7	2.0 ± 0.4	2.0 ± 0.3	2.2 ± 0.3	1.2 ± 0.2	1.4 ± 0.2	1.7 ± 0.3
PGI_2 7.5 ng/kg/min	1.6 ± 0.5	1.3 ± 0.3	1.1 ± 0.2	1.6 ± 0.5	1.9 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	1.5 ± 0.3
PGI_2 15 ng/kg/min	2.0 ± 0.5	1.0 ± 0.4	1.5 ± 0.4	1.5 ± 0.3	2.2 ± 0.5	1.5 ± 0.3	1.1 ± 0.2	1.2 ± 0.5
PGI_2 30 ng/kg/min	2.3 ± 0.5	1.0 ± 0.2	1.7 ± 0.3	1.6 ± 0.5	2.2 ± 0.4	1.7 ± 0.3	1.7 ± 0.3	1.8 ± 0.3

TABLE 3.3

Parameter	Time (minutes) 0	30	60	90	120	150	180	210	240
Plasma Renin Activity (ng/ml/hr)									
Buffer	1.7 ± 0.5		1.7 ± 0.6		1.7 ± 0.5		1.6 ± 0.7		1.6 ± 0.6
PGI_2 7.5 ng/kg/min	1.9 ± 0.5		2.0 ± 0.6		1.9 ± 0.4		1.9 ± 0.3		1.6 ± 0.3
PGI_2 15 ng/kg/min	1.6 ± 0.4		1.7 ± 0.3		1.5 ± 0.3		1.4 ± 0.4		1.5 ± 0.2
PGI_2 30 ng/kg/min	1.4 ± 0.4		2.1 ± 0.5		1.4 ± 0.3		1.5 ± 0.3		1.6 ± 0.3
6-keto-$PGF_{1\alpha}$ Excretion (ng/min)									
Buffer		2.3 ± 0.3	1.9 ± 0.3	2.0 ± 0.4	2.1 ± 0.4	2.2 ± 0.3	2.1 ± 0.4	2.2 ± 0.4	2.1 ± 0.4
PGI_2 7.5 ng/kg/min		5.4 ± 0.6	5.4 ± 0.7	5.5 ± 0.7	5.6 ± 1.1	7.8 ± 1.3	7.2 ± 1.4	6.1 ± 1.2	5.1 ± 1.2
PGI_2 15 ng/kg/min		4.6 ± 0.7	7.2 ± 1.1	8.4 ± 1.2	9.1 ± 1.3	10.1 ± 1.5	8.6 ± 1.4	10.7 ± 1.9	9.8 ± 1.3
PGI_2 30 ng/kg/min		9.4 ± 2.4	10.7 ± 1.4	16.2 ± 2.9	12.6 ± 2.6	13.2 ± 2.3	14.4 ± 2.9	15.8 ± 2.7	14.5 ± 2.8

TABLE 3.4: Statistical analyses of results.

Parameter	F_1	F_2	F_3
Systemic blood pressure	p <0.1237	p <0.0001	p <0.0026
Heart rate	p <0.3974	p <0.2567	p <0.8515
Renal plasma flow	p <0.5337	p <0.2916	p <0.2178
Glomerular filtration rate	p <0.7750	p <0.1747	p <0.9235
Filtration fraction	p <0.3019	p <0.1643	p <0.1427
Sodium excretion	p <0.2193	p <0.0389	p <0.3820
Potassium excretion	p <0.7810	p <0.0111	p <0.3285
Urine flow	p <0.7055	p <0.2714	p <0.1278
Osmolality excretion	p <0.5923	p <0.2722	p <0.4252
Free water clearance	p <0.5446	p <0.3167	p <0.1373
6-keto-$PGF_{1\alpha}$ excretion	p <0.1138	p <0.0095	p <0.0016

Statistical significance is taken at $p < 0.05$. Significance in F_1 demonstrates that any trend in the parameter over time is different between infusions. Significance in F_2 demonstrates a positive trend in the parameter over time for all infusions. Significance in F_3 demonstrates a difference in the response of the parameter between infusions.

Systemic blood pressure was not different during the low infusion rate of PGI_2 than during infusion of vehicle buffer. Mean systemic blood pressures over the 4 hr periods were 99.6 ± 0.8 mm Hg and 99.8 ± 1.0 mm Hg for the vehicle buffer and 7.5 ng/kg/min PGI_2 infusions respectively.

The decrease in systemic blood pressure during the 15 and 30 ng/kg/min PGI_2 infusions was maximal 60 min after the start of the infusions. At this point, when compared with the equivalent pressure during the vehicle buffer infusion, systemic blood pressure was lowered by 9.3 ± 1.6 and 10.3 ± 2.1 mm Hg during the 15 and 30 ng/kg/min infusion rates of PGI_2 respectively. Statistical comparison demonstrated a significant difference in the blood pressure response between treatments (F_3, $p < 0.0026$). This reflects the much reduced systemic blood pressure at the beginning of the 15 and 30 ng/kg/min PGI_2 infusions compared to the pressure during the vehicle buffer and 7.5 ng/kg/min PGI_2 infusions. The 30 ng/kg/min PGI_2 infusion did not decrease systemic blood pressure to a greater extent than the 15 ng/kg/min PGI_2 infusion which may indicate a steep dose-response curve for PGI_2 on systemic blood pressure. After the first 60 min of the 15 and 30 ng/kg/min PGI_2 infusions, systemic blood pressure showed a progressive increase, approaching values observed during the vehicle buffer infusion. Since the activity of the PGI_2 had been tested, the increase in systemic blood pressure despite continued PGI_2 infusion, could not be attributed to hydrolysis of the PGI_2 infusion solution.

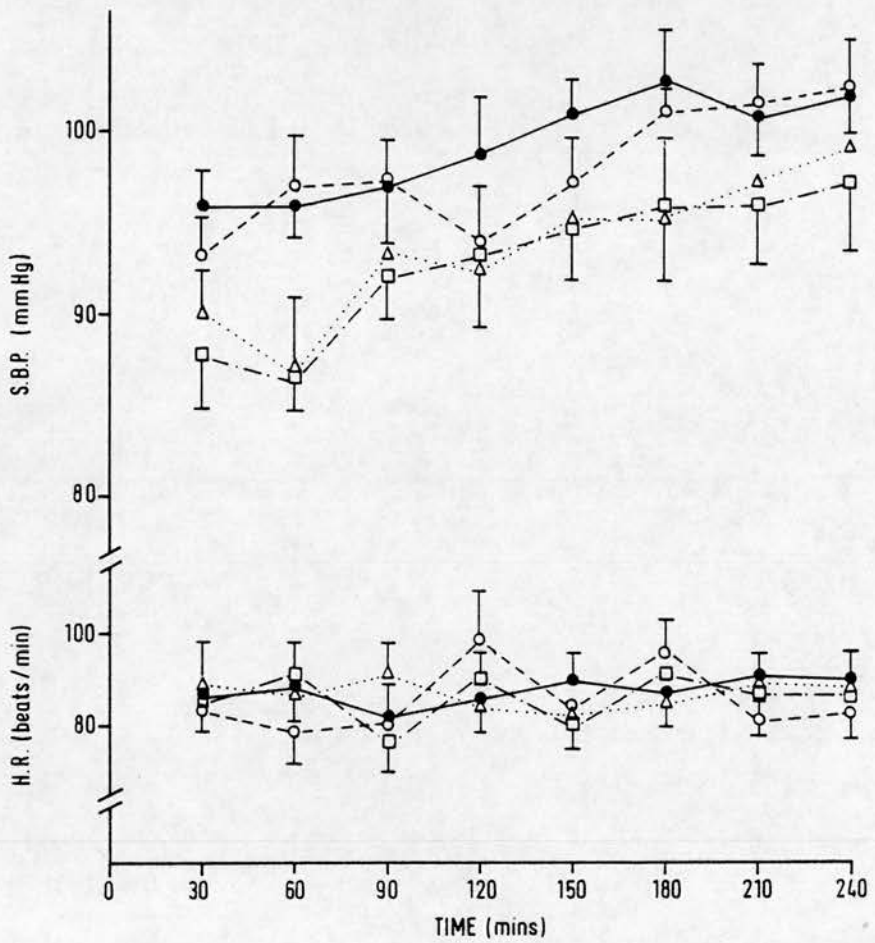


FIGURE 3.1: The effect of intravenous PGI_2 infusion on systemic blood pressure and heart rate.

vehicle buffer;
PGI_2 7.5 ng/kg/min;
PGI_2 15 ng/kg/min;
PGI_2 30 ng/kg/min.

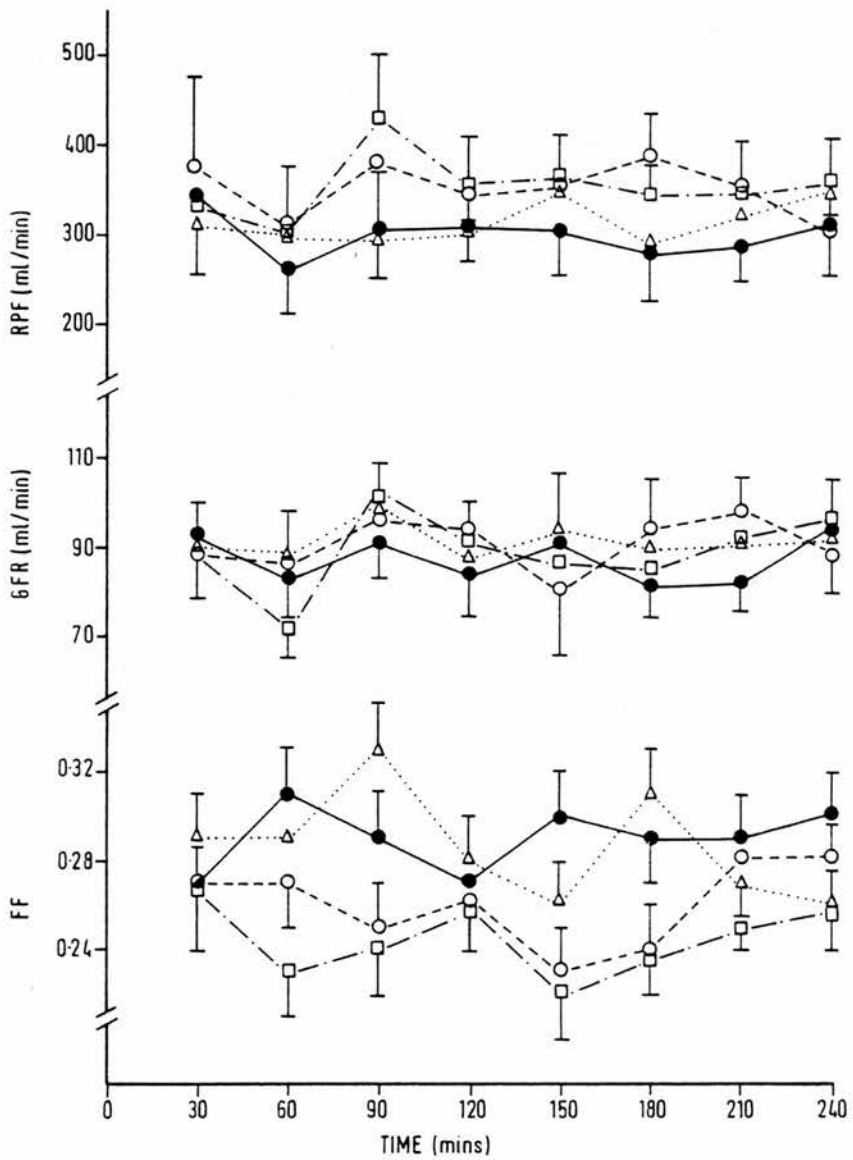


FIGURE 3.2: The effect of intravenous PGI_2 infusion on renal plasma flow, glomerular filtration rate and filtration fraction

vehicle buffer;
PGI_2 7.5 ng/kg/min;
PGI_2 15 ng/kg/min;
PGI_2 30 ng/kg/min.

3.4.2 Heart rate (Fig. 3.1)

No trend was demonstrated in heart rate during infusion of vehicle buffer or during PGI_2 infusion (F_1, $p < 0.3974$; F_2, $p < 0.2567$). There was no significant difference in heart rate between any of the treatments (F_3, $p < 0.8515$). Mean heart rate was 81 ± 4, 86 ± 3, 88 ± 5 and 86 ± 4 beats/min during the vehicle buffer and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively.

3.4.3 Renal plasma flow (Fig. 3.2)

Renal plasma flow did not demonstrate a trend during infusion of vehicle buffer or during PGI_2 infusion (F_1, $p < 0.5337$; F_2, $p < 0.2916$). Mean renal plasma flow rates over the 4 hr infusion periods were 298 ± 28, 351 ± 31, 316 ± 29 and 351 ± 33 ml/min during the vehicle buffer, and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively. Although renal plasma flow was slightly higher during the 7.5 and 30 ng/kg/min PGI_2 infusions than during the vehicle buffer and 15 ng/kg/min PGI_2 infusions, there was no statistically significant difference in renal plasma flow between treatments (F_3, $p < 0.2178$).

3.4.4 Glomerular filtration rate (Fig. 3.2)

Glomerular filtration rate did not demonstrate a trend during infusion of vehicle buffer or during PGI_2 infusion (F_1, $p < 0.7750$; F_2, $p < 0.1747$). There was a transient fall in glomerular filtration rate 60 min after the start of the 30 ng/kg/min PGI_2 infusion which was presumably consequent to the fall in systemic blood pressure. There was no statistically significant difference in glomerular filtration rate between treatments (F_3, $p < 0.9235$). Mean glomerular filtration rates over the 4 hr infusion periods were 88 ± 7, 92 ± 9, 92 ± 7 and 86 ± 6 ml/min

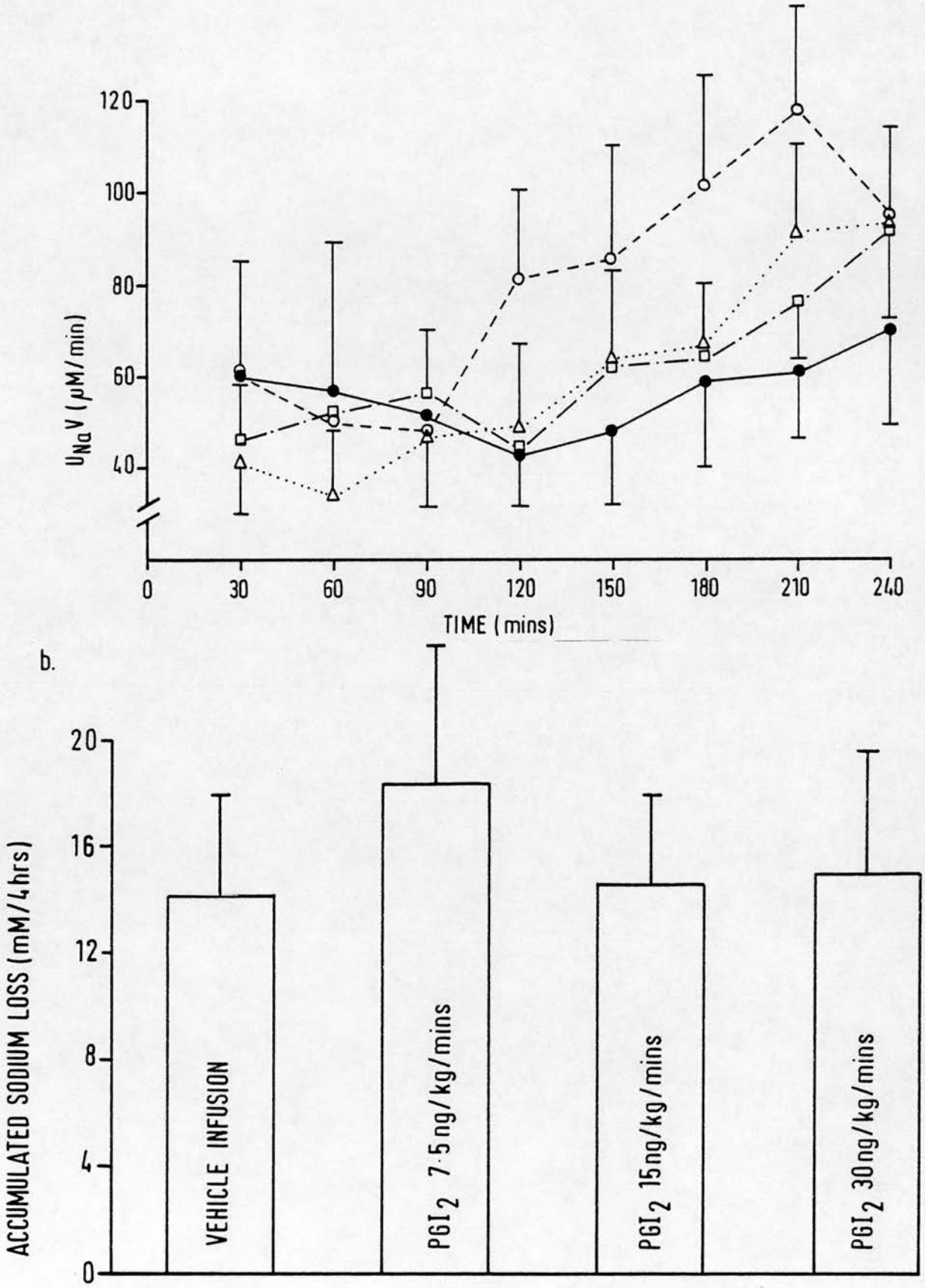


FIGURE 3.3: The effect of intravenous PGI_2 infusion on sodium excretion and accumulated sodium loss.

vehicle buffer; PGI_2 7.5 ng/kg/min

PGI_2 15 ng/kg/min; PGI_2 30 ng/kg/min

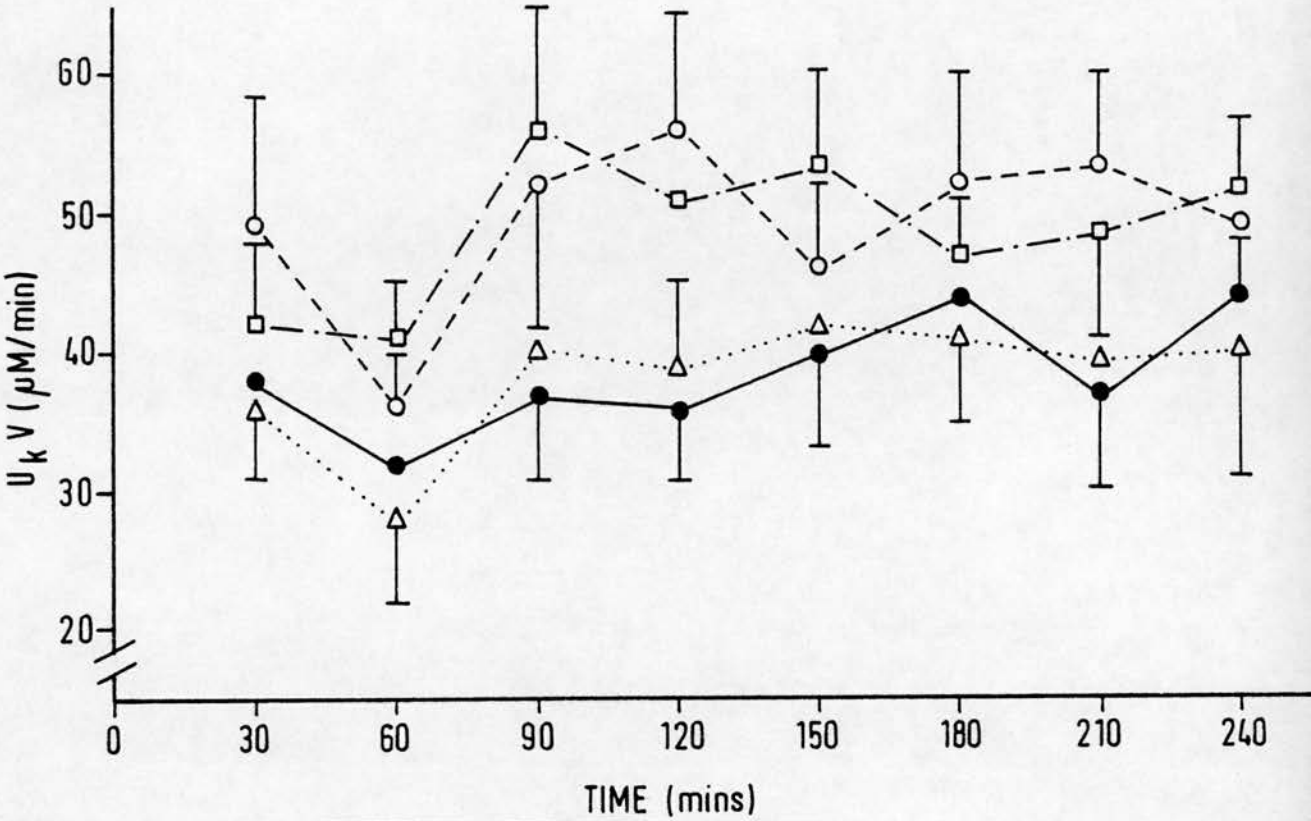
during the vehicle buffer, and 7.5, 15 and 30 ng/kg/min PGI_2 infusion rates, respectively.

3.4.5 Filtration fraction (Fig. 3.2)

Filtration fraction fluctuated markedly throughout the infusion periods, particularly during PGI_2 infusion. However, no general trend was observed during any infusion (F_1, $p < 0.3019$; F_2, $p < 0.1643$). Mean values for filtration fraction over the 4 hr infusion periods were 0.29 ± 0.02, 0.26 ± 0.02, 0.28 ± 0.03 and 0.24 ± 0.02 for the vehicle buffer, and 7.5, 15 and 30 ng/kg/min PGI_2 infusion rates respectively. Filtration fraction was lower during the 7.5 and 30 ng/kg/min PGI_2 infusions due to the small increase in renal plasma flow observed during these infusions, without a similar increase in glomerular filtration rate. There was, however, no statistical difference in filtration fraction between treatments (F_3, $p < 0.1427$).

3.4.6 Urinary sodium excretion (Fig. 3.3)

Sodium excretion demonstrated a small progressive increase towards the end of the vehicle buffer infusion which may be related to the similar progressive increase observed in systemic blood pressure. Sodium excretion also increased during all concentrations of PGI_2 infusion (F_1, $p<0.2193$; F_2, $p < 0.0389$). Although the large standard deviations prevented any statistically significant difference to be observed between treatments (F_3, $p < 0.3820$), the demonstrably larger and earlier increase in sodium excretion during the 7.5 ng/kg/min PGI_2 infusion was thought to be of consequence. Expressed as accumulated sodium loss over the 4 hr period (Fig. 3.3), net sodium loss during the 7.5 ng/kg/min PGI_2 infusion was 18.6 ± 5 mM/4 hr compared with



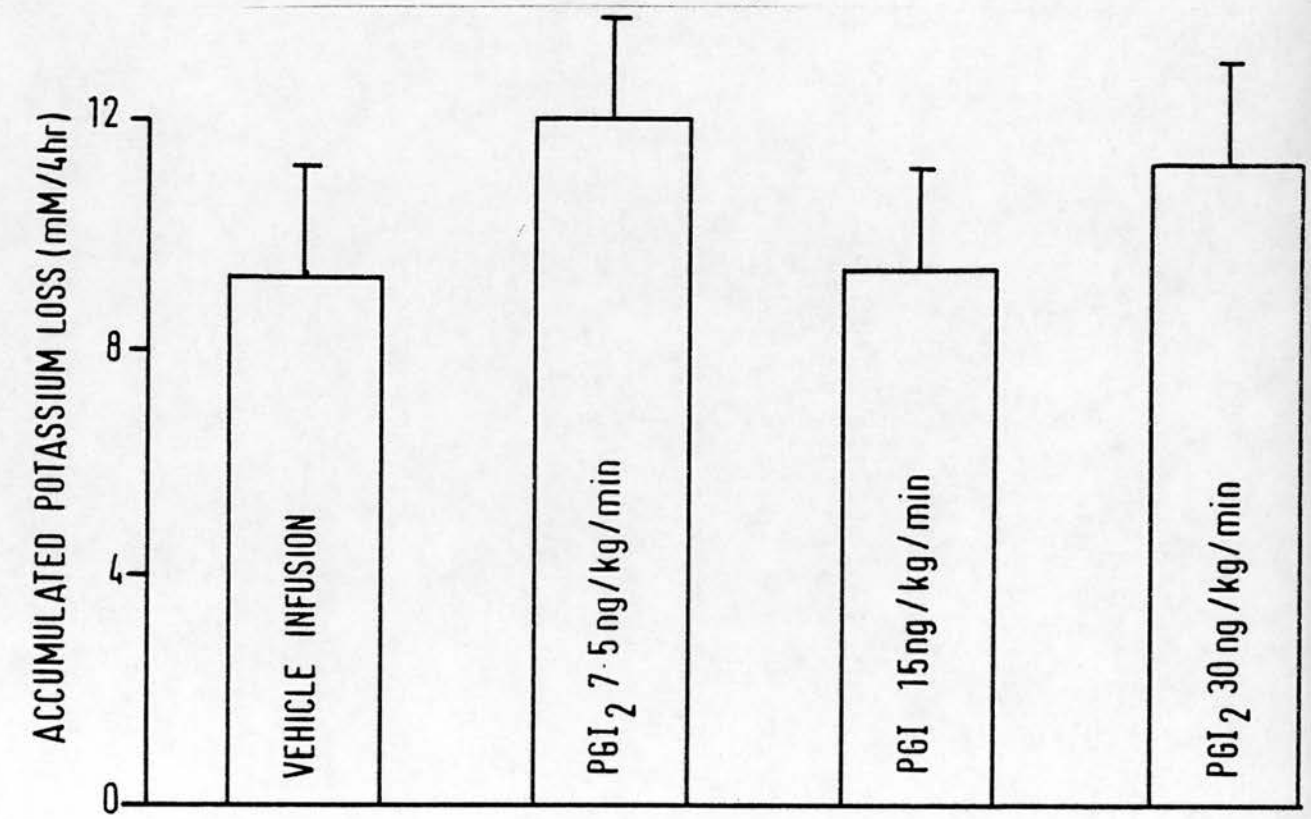


FIGURE 3.4: The effect of intravenous PGI_2 infusion on potassium excretion and accumulated potassium loss.

●——● vehicle buffer; ○– – –○ PGI_2 7.5 ng/kg/min

Δ· · · · ·Δ PGI_2 15 ng/kg/min; □– · – ·□ PGI_2 30 ng/kg/min

13.2 ± 4.8, 14.6 ± 3.4 and 15.0 ± 2.6 mM/4 hr during the vehicle buffer, and 15 and 30 ng/kg/min PGI_2 infusions respectively.

3.4.7 Urinary potassium excretion (Fig. 3.4)

Potassium excretion tended to fluctuate throughout the infusions. However, statistical analysis demonstrated a similar significant trend during all four infusions (F_1, $p < 0.7810$; F_2, $p < 0.0111$). This trend was a small maintained increase in potassium excretion observed 90 min after the start of each infusion. Although potassium excretion was higher during the 7.5 and 30 ng/kg/min PGI_2 infusions, there was no statistically significant difference in potassium excretion between treatments (F_3, $p < 0.3285$). Expressed as accumulated potassium loss over the 4 hr periods (Figure 3.4), net potassium loss was 12.2 ± 2.6 and 11.6 ± 2.4 mM/4 hr for the 7.5 and 30 ng/kg/min PGI_2 infusions compared with 9.6 ± 2.1 and 9.7 ± 2.3 mM/4 hr for the vehicle buffer and 15 ng/kg/min PGI_2 infusions. The slightly higher potassium excretion during the 7.5 and 30 ng/kg/min PGI_2 infusions may be related to the similarly elevated renal plasma flow observed during these infusions.

3.4.8 Urine flow (Fig. 3.5)

Urine flow did not demonstrate any trend during infusion of vehicle buffer or during PGI_2 infusion (F_1, $p < 0.7055$; F_2, $p < 0.2714$). There was a transient fall in urine flow 60 min after the start of the 15 and 30 ng/kg/min PGI_2 infusions. This was most likely a consequence of the decreased systemic blood pressure. There was no statistically significant difference in urine flow between treatments (F_3, $p < 0.1278$). Mean urine flow rates over the 4 hr infusion periods were 3.6 ± 0.3, 3.6 ± 0.2, 3.2 ± 0.3 and 3.6 ± 0.4 ml/min during the vehicle buffer and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively.

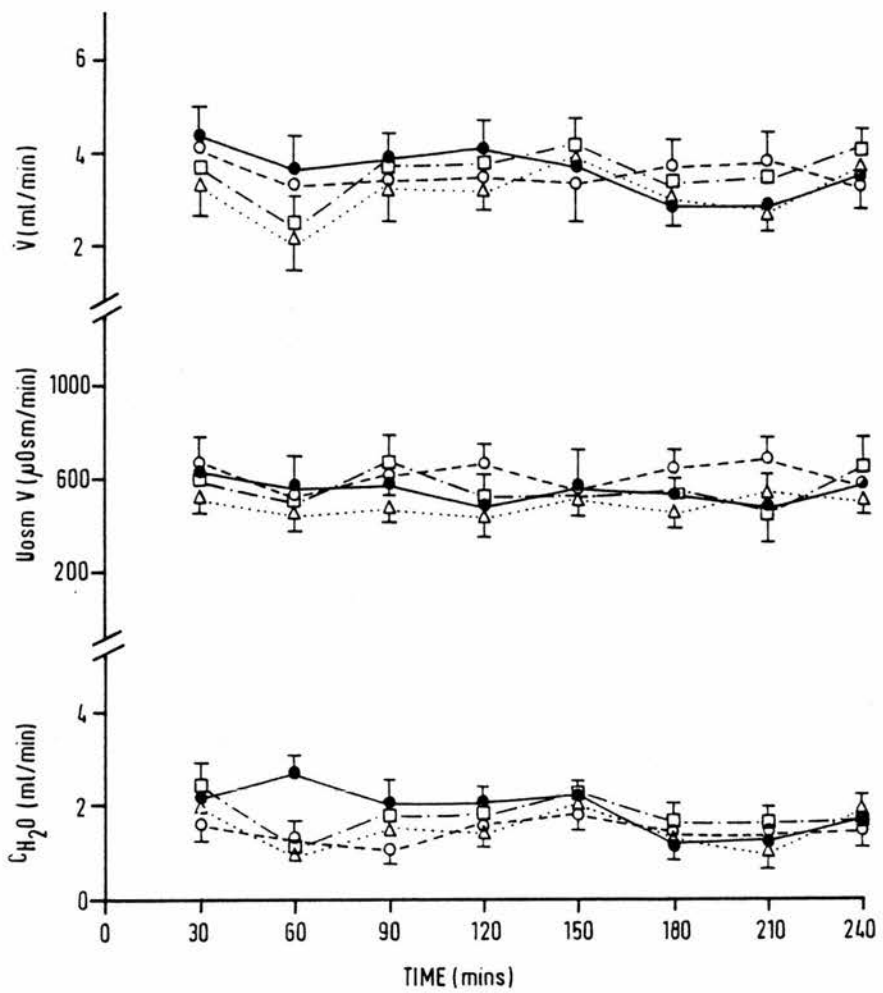


FIGURE 3.5: The effect of intravenous PGI_2 infusion on urine flow, osmolality excretion and free water clearance.

vehicle buffer;
PGI_2 7.5 ng/kg/min;
PGI_2 15 ng/kg/min;
PGI_2 30 ng/kg/min.

3.4.9 Solute (osmolality) excretion (Fig 3.4)

There was no trend in solute excretion during vehicle buffer or PGI_2 infusion (F_1, $p < 0.5923$; F_2, $p < 0.2722$), nor was there any difference in solute excretion between treatments (F_3, $p < 0.4252$). Mean osmolality excretion rates over the 4 hr infusion periods were 548 ± 40, 614 ± 43, 509 ± 49 and 550 ± 41 µosm/min during the vehicle buffer and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively.

3.4.10 Free water clearance (Fig. 3.5)

There was no trend in free water clearance during vehicle buffer or PGI_2 infusion (F_1, $p < 0.5445$; F_2, $p < 0.3167$), nor was there any difference in free water clearance between treatments (F_3, $p < 0.1373$). Mean free water clearance rates over the 4 hr infusion periods were 1.9 ± 0.2, 1.5 ± 0.2, 1.5 ± 0.3 and 1.7 ± 0.4 ml/min during the vehicle buffer and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively.

3.4.11 Plasma renin activity (Fig. 3.6)

Plasma renin activity did not differ from the control value of 1.7 ± 0.5 ng/ml/hr during the 4 hr infusion of vehicle buffer. PGI_2 infusion had remarkably little effect on plasma renin activity. Neither the 7.5 nor the 15 ng/kg/min PGI_2 infusions altered plasma renin activity from their control values of 1.9 ± 0.5 and 1.6 ± 0.4 ng/ml/hr respectively. Plasma renin activity increased transiently from 1.4 ± 0.4 to 2.1 ± 0.5 ng/ml/hr 60 min after the start of the 30 ng/kg/min PGI_2 infusion, but this increase was not statistically significant. Plasma renin activity was returned to control levels by 120 min and remained at control levels throughout the rest of the 30 ng/kg/min PGI_2 infusion.

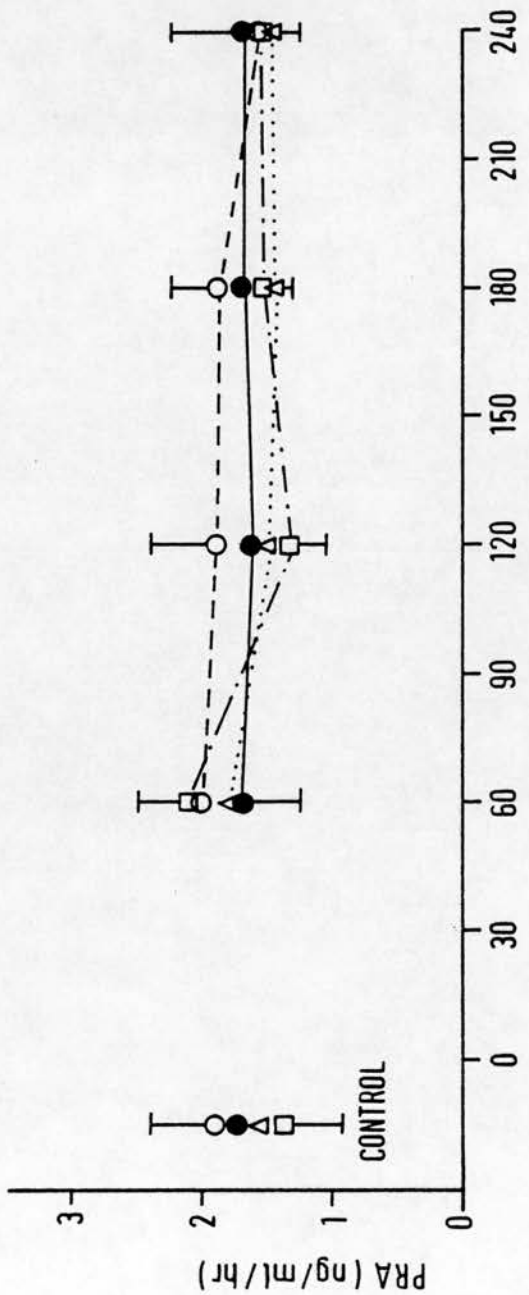


FIGURE 3.6: The effect of intravenous PGI_2 infusion on plasma renin activity.

● vehicle buffer; ○ – – –○ PGI_2 7.5 ng/kg/min
Δ · · · · · Δ PGI_2 15 ng/kg/min; □ – · – · □ PGI_2 30 ng/kg/min

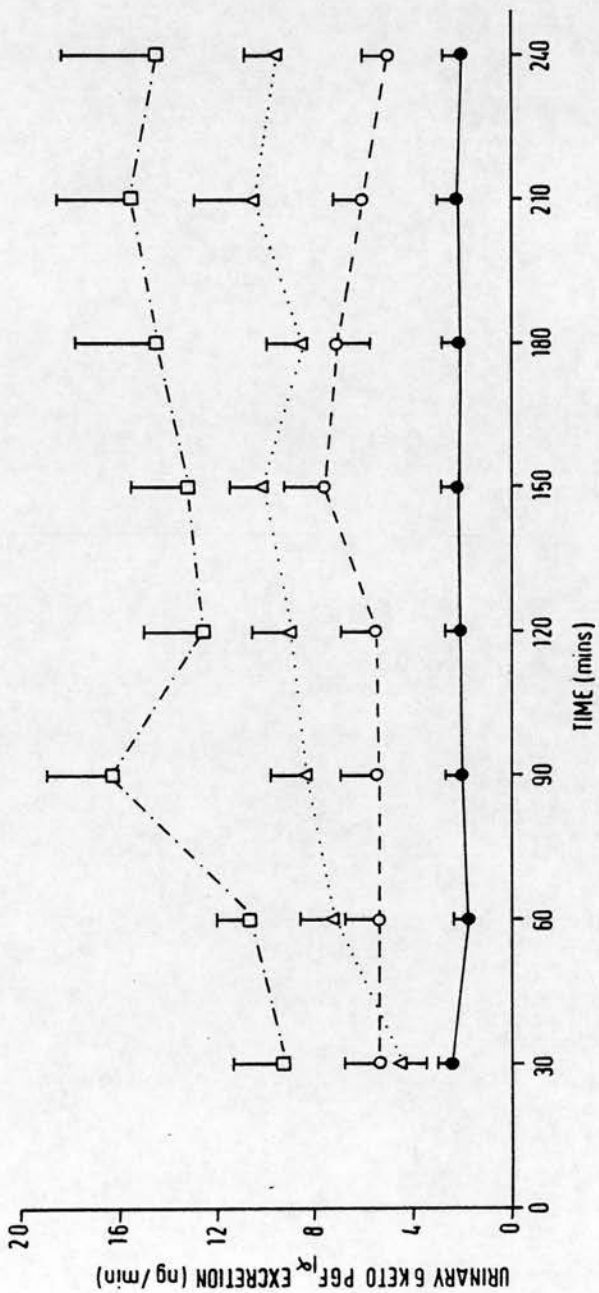


FIGURE 3.7: Urinary 6-keto-$PGF_{1\alpha}$ excretion during intravenous PGI_2 infusion.

● vehicle buffer; O– – –O PGI_2 7.5 ng/kg/min

Δ· · · · · Δ PGI_2 15 ng/kg/min; □– · – · □ PGI_2 30 ng/kg/min

3.4.12 Urinary 6-keto-PGF$_{1\alpha}$ excretion (Fig. 3.7)

6-keto-PGF$_{1\alpha}$ excretion increased during the first 90 min of the 15 and 30 ng/kg/min PGI_2 infusions before a steady state was achieved. It was difficult to observe a similar trend during the vehicle buffer or 7.5 ng/kg/min PGI_2 infusion although statistical analysis of the urinary 6-keto-PGF$_{1\alpha}$ excretion demonstrated a significant similar trend during all infusions (F_1, $p < 0.1138$; F_2, $p < 0.0095$). Urinary 6-keto-PGF$_{1\alpha}$ excretion increased corresponding to the increasing concentrations of PGI_2 infused and there was a statistically significant difference in the 6-keto-PGF$_{1\alpha}$ excretion between treatments (F^3, $p < 0.0016$).

Since 6-keto-PGF$_{1\alpha}$ excretion only reached a steady state 90-120 min after the start of the PGI_2 infusion, when correlating 6-keto-PGF$_{1\alpha}$ excretion rate with PGI_2 infusion rate, only the last three values for 6-keto-PGF$_{1\alpha}$ excretion during each infusion were incorporated in a mean value. These mean urinary 6-keto-PGF$_{1\alpha}$ excretion rates were 2.1 ± 0.2, 6.1 ± 0.7, 9.7 ± 0.9 and 14.9 ± 1.5 ng/min during the vehicle buffer, and 7.5, 15 and 30 ng/kg/min PGI_2 infusions respectively. These excretion rates represent 2-4% of the originally infused PGI_2.

When the mean 6-keto-PGF$_{1\alpha}$ excretion rates were plotted against the infusion rate of PGI_2, a straight line, indicative of a linear relationship was observed (Figure 3.8). The line demonstrating this relationship had a correlation co-efficient of 0.9938 and was expressed by the equation,

$$y = 0.42x + 2.70$$

In order to estimate an endogenous rate of entry of PGI_2 into the circulation, the mean basal rate of 6-keto-PGF$_{1\alpha}$ excretion was subtracted from each of the mean excretion rates during the PGI_2 infusions.

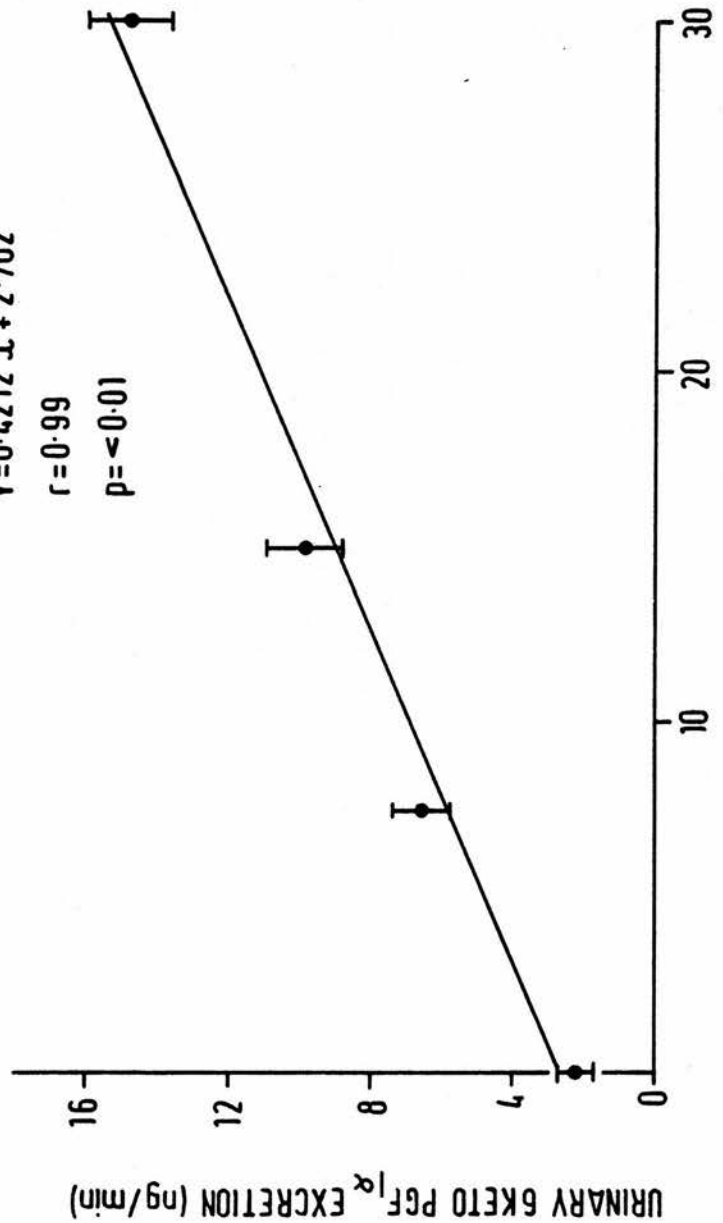


FIGURE 3.8: Linear relationship between PGI_2 infusion rate and urinary excretion of $PGF_{1\alpha}$.

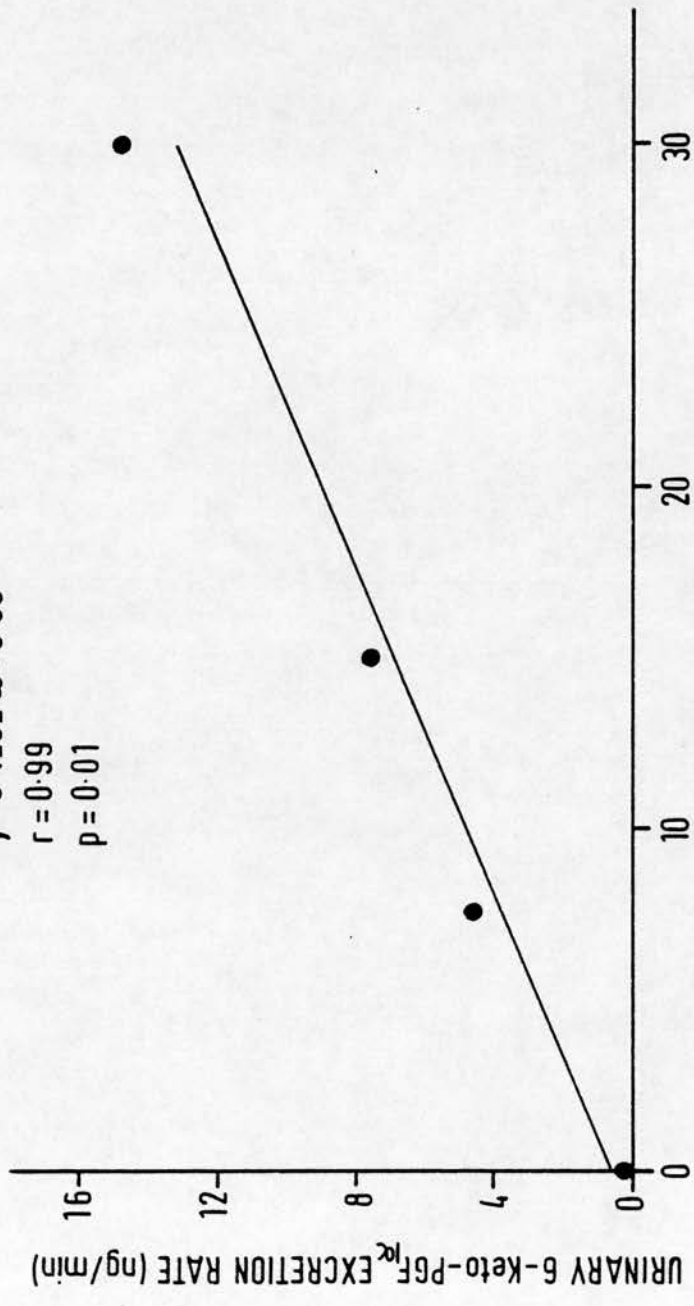


FIGURE 3.9: Relationship between PGI_2 infusion rate and urinary 6-keto-$PGF_{1\alpha}$ excretion following subtraction of endogenous 6-keto-$PGF_{1\alpha}$ excretion and with the line constrained to pass through the origin.

The increments in 6-keto-$PGF_{1\alpha}$ excretion rates were then plotted against PGI_2 infusion rate and the line drawn constrained to pass through the origin (Figure 3.9). In drawing such a graph, it was assumed that the linear relationship between the rate of PGI_2 infused and the quantity of metabolite excreted, existed for PGI_2 infusion below 7.5 ng/kg/min. When constrained through the origin, the line had a correlation coefficient of 0.9923 and was expressed by the equation,

$$y = 0.42x + 0.66$$

From this line, the endogenous 6-keto-$PGF_{1\alpha}$ excretion rate could be equated with a rate of entry of PGI_2 into the circulation. This estimation of the rate of entry into the circulation represents a maximum value since contribution to the urine of 6-keto-$PGF_{1\alpha}$ derived from PGI_2 of renal origin cannot be excluded. On this basis, the calculated endogenous rate of entry of PGI_2 into the circulation of the conscious dog had a maximal value of 4 ng/kg/min. Taking average body weight as 20 kg, this represents a rate of entry of 80 ng/min.

3.5 Discussion

It has been suggested that PGI_2 may contribute to the control of systemic blood pressure, either as a circulating hormone, possibly released from the lung (Gryglewski, *et al.*, 1978), or as a local hormone synthesised by the vascular endothelium (Armstrong *et al.*, 1978). In the present study, intravenous infusion of PGI_2 at 7.5 ng/kg/min had no effect on systemic blood pressure and infusion of PGI_2 at 15 or 30 ng/kg/min caused only transient decreases in systemic blood pressure. When basal urinary 6-keto-$PGF_{1\alpha}$ excretion rate was equated

with a rate of entry of PGI_2 into the circulation, the estimated value was 4 ng/kg/min. This rate of formation of PGI_2 is well below the lowest intravenous infusion rate of PGI_2 which had no effect on systemic blood pressure. It is therefore unlikely that endogenous PGI_2 contributes to the control of systemic blood pressure under normal conditions in the conscious dog. The lack of sensitivity of the vasculature to exogenous infusion of PGI_2 suggests that even when PGI_2 synthesis is stimulated, it does not play a major role as a circulating hormone in the control of systemic blood pressure. This conclusion is supported by the demonstration that administration of PGI_2-specific antibodies to normotensive or hypertensive rats fails to have any effect on systemic blood pressure (Pace-Asciak, Carrara, Levine and Nicolaou, 1980).

The reversal of the hypotension observed during the early period of the 15 and 30 ng/kg/min PGI_2 infusions may have been due, at least in part, to baroreceptor reflex since this compensatory mechanism is responsive to decreases in mean systemic blood pressure (Ead, Green and Neil, 1952). There may also have been a centrally mediated reflex increase in sympathetic activity resulting in more noradrenaline being released at the adrenergic terminals. Prolonged infusion of PGE_2 into the renal artery of conscious dogs results in an increase in systemic blood pressure which can be positively correlated to an increase in plasma renin activity (Hockel and Cowley, 1980). Since PGI_2 is a more potent renin secretagogue than PGE_2 (Gerber *et al.*, 1979), it could be postulated that a similar mechanism was operating to prevent the prolonged PGI_2 infusion resulting in a sustained hypotension. However, it was apparent from the results of the study that this was not the case. The 15 ng/kg/min PGI_2 infusion had no effect on plasma renin activity and the 30 ng/kg/min PGI_2 infusion resulted in only a small increase in

plasma renin activity, even though both these PGI_2 infusions decreased systemic blood pressure.

The lack of effect of intravenously infused PGI_2 on renin release, even in concentrations which lower systemic blood pressure, is surprising in view of the amount of evidence supporting an important role for PGI_2 in the control of renin release. Most of the previous studies demonstrating the effect of PGI_2 to increase renin release have been performed on anaesthetised animal models or on isolated tissue preparations. It is possible that under surgically traumatised conditions, the juxtaglomerular cells become more sensitive to the renin secretory activity of PGI_2 and that in the conscious, untraumatised animal, PGI_2 may not be so potent in stimulating renin release.

There may also be a species difference in the effect of PGI_2 to stimulate renin release. A 4 hr intravenous infusion of 8 ng/kg/min PGI_2 into normal humans, which had no effect on mean arterial pressure, resulted in an elevation in plasma renin activity which was sustained for the duration of the infusion (Fitzgerald, Hossman, Hummerich and Konrads, 1980). Intravenous infusion of 10 ng/kg/min PGI_2 into conscious rats had no effect on plasma renin activity but infusion of 100 ng/kg/min PGI_2 resulted in a doubling of plasma renin activity (Weeks and Compton, 1979). It would seem, therefore, that PGI_2 is more potent in stimulating renin release in normal humans than in conscious dogs or rats.

Although the only detectable change in plasma renin activity in the conscious dogs was a small increase 30 min after the start of the 30 ng/kg/min PGI_2 infusion, the blood samples for the plasma renin activity determinations were taken from the carotid artery. It is possible that a small increase in renin release may have occurred within the

kidney during PGI_2 infusion, without this increase being detected in arterial blood.

PGI_2 infusion had no dose-dependent action to increase renal plasma flow. Small increases in renal plasma flow were observed during the 7.5 and 30 ng/kg/min PGI_2 infusions but the 15 ng/kg/min PGI_2 infusion had no effect on renal plasma flow. PGI_2 infusion had no effect on glomerular filtration rate even during the 7.5 and 30 ng/kg/min infusions when renal plasma flow was slightly elevated. This indicates that the afferent and efferent arterioles had been dilated to the same extent. The lack of a larger dose-dependent effect of intravenous PGI_2 on the renal vasculature suggests that circulating PGI_2 does not influence renal haemodynamics. However, PGI_2 is synthesised by the glomeruli (Hassid *et al.*, 1979) and the arteries and arterioles in the cortex (Terragno *et al.*, 1978) and may act as a local hormone in the control of renal blood flow.

Studies using prostaglandin synthesis inhibitors to elucidate the role of renal prostaglandins in the control of renal haemodynamics have given conflicting results. In the anaesthetised dog, indomethacin administration resulted in a decrease in renal blood flow and glomerular filtration rate (Lonigro *et al.*, 1973; Feigen, Klainer, Chapwick and Kadowitz, 1976). More recent studies in conscious dogs have demonstrated that administration of either indomethacin or meclofenamate has no effect on renal blood flow or glomerular filtration rate unless the renin-angiotensin system is stimulated by sodium depletion (Blasingham *et al.*, 1980; DeForrest *et al.*, 1980). Under these conditions, administration of indomethacin or meclofenamate decreases both renal plasma flow and glomerular filtration rate. The lack of effect of indomethacin on renal blood flow in the normal dog, and the demonstration that

intravenously infused PGI_2 has very little effect on renal plasma flow, suggests that in the conscious animal when the extracellular fluid volume is fully expanded and the activity of the renin-angiotensin system is low, PGI_2 does not play a significant role in the control of renal haemodynamics.

The pattern of electrolyte excretion was altered by intravenous PGI_2 infusion. The greatest increase in sodium excretion was observed during the 7.5 ng/kg/min PGI_2 infusion, the 15 and 30 ng/kg/min PGI_2 infusions demonstrating very little effect on sodium excretion until the end of the infusions. The decrease in systemic blood pressure during the first part of the 15 and 30 ng/kg/min PGI_2 infusions may have been preventing an early increase in sodium excretion. It would seem therefore that low concentrations of PGI_2 which do not affect systemic blood pressure, can exert an influence on sodium excretion. Thus the endogenous rate of entry of PGI_2 into the circulation (4 ng/kg/min), which is too low to affect systemic blood pressure or renal plasma flow, may be involved in the control of sodium excretion.

It is possible that the natriuretic action of the 7.5 ng/kg/min PGI_2 infusion is a consequence of changes in renal blood flow not detected by the present methodology. Renal vasodilatation in the kidney can cause natriuresis independent of changes in glomerular filtration rate, either as a result of decreases in peritubular capillary oncotic pressures or increases in hydrostatic pressure (Earley and Friedler, 1965; Schrier and De Wardener, 1971). PGI_2 can also cause redistribution of renal blood flow from the outer cortex to the juxtamedullary region (Gerber *et al.*, 1978a; Bolger *et al.*, 1978). This may result in natriuresis either by reducing the renal medullary osmotic gradient as a result of a washout effect (Earley and Friedler, 1966) or by increasing preferentially

perfusion of the inner cortical nephrons which may have a small intrinsic capacity to reabsorb sodium (Stein, Osgood and Kunan, 1976). These alterations in the Starling forces in the peritubular capillary circulation caused by a redistribution of intrarenal blood flow, result in a decrease in proximal tubular reabsorption of sodium (Lewy and Windhanger, 1968).

There was no change in free water clearance during the 7.5 ng/kg/min PGI_2 infusion suggesting that the effect of the PGI_2 on sodium reabsorption was primarily on the proximal tubule. If PGI_2 were to have decreased sodium reabsorption in the distal tubule, free water clearance would have also decreased (Seldin, Eknoyan, Suki and Rector, 1966).

The close correlation between the increase in potassium excretion and renal plasma flow in the 7.5 and 30 ng/kg/min PGI_2 infusions also suggests that the natriuresis observed during PGI_2 infusion is a consequence of renal vasodilation and decreased proximal sodium reabsorption. Potassium is completely reabsorbed by the proximal tubule and potassium in the urine is derived from distal tubular secretion. Potassium competes with hydrogen ions for an exchange mechanism with sodium in the distal tubular lumen. A decrease in proximal tubule sodium reabsorption would result in more sodium being available in the distal tubule to enter into an exchange mechanism with potassium. Assuming there was no change in ascending limb function, this could result in an increase in potassium excretion.

PGI_2 has been demonstrated to inhibit sodium transport in isolated rabbit cortical collecting tubules (Iino and Brenner, 1981). An increase in solute delivery to the distal tubule and a decrease in distal tubule sodium reabsorption has been demonstrated during PGI_2 infusion in anaesthetised dogs (Gullner *et al.*, 1980). However, in that study renal blood flow was not measured and the observed effects on sodium

reabsorption could have been consequent to changes in renal haemodynamics.

It seems likely that the increase in sodium excretion observed during the 7.5 ng/kg/min PGI_2 infusion is due to decreased proximal tubule reabsorption as a result of redistribution of intrarenal blood flow although this could not be proved conclusively with the present methodology.

Over the four-fold PGI_2 infusion range, a linear relationship was observed between the quantity of PGI_2 intravenously infused and urinary excretion rate of 6-keto-$PGF_{1\alpha}$. This demonstrates that although 6-keto-$PGF_{1\alpha}$ is only one of several metabolites of PGI_2 to be excreted in the urine, its measurement does reliably reflect circulating PGI_2 levels.

The mean basal 6-keto-$PGF_{1\alpha}$ excretion rate of 2.1 ng/min has been confirmed by a study in which the urinary 6-keto-$PGF_{1\alpha}$ excretion rate in anaesthetised dogs was approximately 2 ng/min, as measured by radioimmunoassay (Wilson, Loadholt, Privitera and Halushka, 1982). Basal urinary 6-keto-$PGF_{1\alpha}$ excretion in humans is much lower than in dogs. Measurement by radioimmunoassay of 6-keto-$PGF_{1\alpha}$ in the urine of a group of female subjects gave a mean excretion rate of 0.62 ng/min (Patrono, Ciabattoni, Cinotti, Pugliese, Maseri and Chierchia, 1979) whilst in another group of subjects, measurement by gas chromatography-mass spectrometry gave a mean urinary 6-keto-$PGF_{1\alpha}$ excretion rate of 0.2 ng/min (Fischer, Scherer and Weber, 1983).

Although there has been wide variation in the reported plasma concentration of 6-keto-$PGF_{1\alpha}$ in humans, recent observations using sensitive RIA and GC-MS have indicated that the plasma concentration of 6-keto-$PGF_{1\alpha}$ is very low. Levels of approximately 5 pg/ml have

been detected in human plasma by RIA (Siess and Dray, 1982) and levels of less than 3 pg/ml were detected by GC-MS (Blair, Barrow, Waddell, Lewis and Dollery, 1982). Measurement by GC-MS of 6-keto-$PGF_{1\alpha}$ in the plasma of conscious dogs demonstrated levels of 70-80 pg/ml (Jackson, Gerkens, Brash and Branch, 1982). These levels were much higher than those found in the plasma of humans.

It would seem therefore that there is a species difference between humans and dogs in the amount of 6-keto-$PGF_{1\alpha}$ present in the urine and plasma. The urinary concentration of 6-keto-$PGF_{1\alpha}$ in dogs is approximately 10-fold higher than that in humans whilst the plasma concentration of 6-keto-$PGF_{1\alpha}$ in dogs is approximately 40-fold higher than that in humans. This may represent a higher rate of synthesis of PGI_2 in the dog and/or a difference in the metabolism of PGI_2.

The estimated rate of entry of PGI_2 into the circulation of the conscious dog was 4 ng/kg/min. In man, measurement by GC-MS of the urinary excretion rates of 2,3-dinor-6-keto-$PGF_{1\alpha}$ and 15-keto-13,14-dihydro-2,3-dinor-6-keto-$PGF_{1\alpha}$ during PGI_2 infusion gave an estimated rate of entry of PGI_2 into the circulation of 0.08 - 0.1 ng/kg/min (FitzGerald, Brash, Falardeau and Oates, 1981). This represents an approximately 40-fold difference between humans and dogs in the rate of entry of PGI_2 into the circulation and suggests that there is a higher rate of synthesis of PGI_2 in the dog.

After subtraction of the basal rate, the urinary excretion rates of 6-keto-PGF_{1_α} during PGI_2 infusion represented 2-4% of the infused PGI_2. Infusion of large quantities of PGI_2 with a small quantity of radiolabel over a short period of time into humans resulted in approximately 15% of the radioactivity being recovered as urinary 6-keto-$PGF_{1\alpha}$ (Rosenkranz *et al.*, 1980). This suggests that less PGI_2 is excreted

as unchanged 6-keto-$PGF_{1\alpha}$ in dogs than in humans and indicates a species difference in the metabolism of PGI_2. 6-keto-$PGF_{1\alpha}$ has been identified as the major metabolite in human plasma during intravenous PGI_2 infusion (Rosenkranz, Fischer and Frolich, 1981), but a recent study in dogs has demonstrated that following a bolus intravenous injection, plasma 6-keto-$PGF_{1\alpha}$ declines rapidly and the major plasma metabolite is 2,3-dinor, -13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$ (Taylor, Shebuski and Sun, 1983). Thus, whilst urinary 6-keto-$PGF_{1\alpha}$ linearly reflects circulating PGI_2 levels, it would seem that, in the dog, 6-keto-$PGF_{1\alpha}$ is not the main urinary or plasma metabolite of PGI_2. This may be indicative of a greater capacity for 15-hydroxydehydrogenation and β-oxidation of PGI_2 in the dog than is apparent in man.

The estimation of the rate of entry of PGI_2 into the circulation represents a maximum value for two reasons. Firstly, the PGI_2 may be hydrolysed in the organ of production and may be entering the circulation as 6-keto-$PGF_{1\alpha}$. Secondly, basal urinary 6-keto-$PGF_{1\alpha}$ excretion may represent not only 6-keto-$PGF_{1\alpha}$ from the systemic circulation but also 6-keto-$PGF_{1\alpha}$ derived from PGI_2 of renal origin. The demonstration that 6-keto-$PGF_{1\alpha}$, unlike PGE_2 and $PGF_{2\alpha}$, does not enter the urine by an organic acid pathway, but is freely filtered at the glomerulus (Rosenkranz *et al.*, 1981a) suggested that the 6-keto-$PGF_{1\alpha}$ in the urine was derived solely from the systemic circulation. However, there is also evidence to suggest that PGI_2 is synthesised by the epithelial and mesangial cells of the glomerulus (Kreisberg , 1982; Scharschmidt and Dunn, 1983). This PGI_2 would probably enter the urine at the glomerulus without being subjected to 15-hydroxydehydrogenation or β-oxidation. It is also possible that 6-keto-$PGF_{1\alpha}$ could enter the

tubular lumen at another site, distinct from the organic acid pathway. Care must therefore be taken when interpreting urinary 6-keto-$PGF_{1\alpha}$ data, particularly when relating it to changes in circulating PGI_2 levels, since it cannot always be assumed that the contribution of renal 6-keto-$PGF_{1\alpha}$ to the urine remains constant. This may be particularly important under conditions of stimulated renin release where urinary 6-keto-$PGF_{1\alpha}$ excretion may reflect predominantly renal PGI_2 production.

A further consideration in the interpretation of urinary 6-keto-$PGF_{1\alpha}$ data is that a change in urinary 6-keto-$PGF_{1\alpha}$ may not only reflect a change in PGI_2 synthesis, but may also represent a shift in the metabolic pattern of PGI_2. Thus an increase in 6-keto-$PGF_{1\alpha}$ excretion may be due to an increase in PGI_2 synthesis or may be the consequence of reduced enzymatic metabolism of PGI_2 resulting in an increased excretion of unchanged 6-keto-$PGF_{1\alpha}$. Were this to be the case, it would obviously be advantageous to monitor more than one metabolite of PGI_2. However, over a physiological range of PGI_2 production, it seems unlikely that there would be large shifts in the metabolic pattern.

In conclusion, the present study has demonstrated that over the 4-fold PGI_2 infusion range 7.5 - 30 ng/kg/min, urinary 6-keto-$PGF_{1\alpha}$ linearly reflects the circulating PGI_2 and therefore within this range, its measurement provides a useful and reliable indication of the rate of PGI_2 synthesis. The estimated maximum endogenous rate of entry of PGI_2 into the circulation derived from the urinary 6-keto-$PGF_{1\alpha}$ measurements was 4 ng/kg/min. This value was considerably lower than the lowest intravenous PGI_2 infusion rate of 7.5 ng/kg/min which had no effect on systemic blood pressure. It therefore seems unlikely that PGI_2 is present in sufficient quantities in the systemic circulation

to be acting as a circulating hormone in the control of systemic blood pressure. It is also unlikely that the 4 ng/kg/min rate of entry of PGI_2 into the circulation is sufficient to be exerting an influence on renal haemodynamics since intravenous infusion of PGI_2 up to 30 ng/kg/min showed no significant effect on renal plasma flow. There remains, however, the possibility that PGI_2 synthesised within the kidney, acts as a local hormone in the control of renal haemodynamics.

The increase in sodium excretion observed during the 7.5 ng/kg/min PGI_2 infusion suggests that low circulating levels of PGI_2 are capable of causing a natriuresis possibly as a result of changes in intra-renal blood flow.

Intravenous PGI_2 infusion had a negligible effect on renin release and although it is still possible that local PGI_2 synthesis plays a role in the control of renin release, it would seem that in the dog, PGI_2 is not such an important renin secretagogue as was previously thought.

This study hasdemonstrated that the basal urinary excretion rate of 6-keto-$PGF_{1\alpha}$ in the dog was higher than that in man although the percentage of PGI_2 excreted as 6-keto-$PGF_{1\alpha}$ was lower. The study has also demonstrated a difference in the effect of PGI_2 on renin release between dog and man. This evidence suggests that there is species difference in the rate of synthesis, pattern of metabolism and physiological response to PGI_2

SECTION IV

The Role of PGI_2 in the Control of Systemic Blood Pressure and Renal Function During Inhibition of the Renin-Angiotensin System with Captopril

4.1 Introduction

Captopril is an important therapeutic agent and, as an inhibitor of angiotensin I converting enzyme, has also proved to be a useful pharmacological tool in delineating the function of the renin-angiotensin system.

Under non-pathological circumstances and when a person or animal has a normal salt intake, administration of a drug which blocks the renin-angiotensin system has an almost imperceptible effect on systemic blood pressure. Thus most previous studies with captopril have been restricted to conditions of sodium depletion or renovascular hypertension when angiotensin II becomes a prime factor in blood pressure control. Chronic administration of captopril to salt depleted conscious dogs results in a sustained reduction in systemic blood pressure, plasma aldosterone concentration and glomerular filtration rate and an increase in renal plasma flow, sodium excretion and plasma renin activity (McCaa, Hall and McCaa, 1978; Hall, Guyton, Smith and Coleman, 1979). Captopril also decreases systemic blood pressure and increases renal plasma flow and plasma renin activity in Goldblatt hypertensive dogs and rats (Zimmerman, Mommsen and Kraft, 1980; Vandongen, Tunney and Martinez, 1981) and in patients with renovascular hypertension (Cody, Tarazi, Bravo and Fo ad, 1978; Aitkinson, Brown, Fraser, Leckie, Lever, Morton and Robertson, 1979). These studies have demonstrated that under conditions where the renin-angiotensin system is stimulated, angiotensin II plays an important role in the control of systemic and renal haemodynamics and sodium excretion. It is possible that angiotensin II exerts this control by a direct vasoconstrictor and anti-natriuretic action since infusion of aldosterone, whose increased release

was previously thought to be responsible for mediating many of the effects of angiotensin II, failed to reverse the effects of chronic captopril administration on systemic blood pressure, renal plasma flow or sodium excretion (Hall *et al.*, 1979).

Studies with the angiotensin II antagonist, saralasin (Hollenberg, Williams, Taub, Ishikawa, Brown and Adams, 1977; Agabiti-Rosei *et al.*, 1979) and the earlier angiotensin I converting enzyme inhibitor teprotide (Kimbrough *et al.*, 1977; Niarchos *et al.*, 1979), failed to produce consistent evidence that angiotensin II was important in the control of systemic and renal haemodynamics in normal, salt replete humans or animals. In contrast to these results, however, captopril administration has resulted in a decrease in systemic blood pressure and an increase in renal plasma flow in sodium replete conscious rats (Johnston, Bernard, Perrin, Arbeit, Liberthal and Levinsky, 1981) and dogs (Zimmerman *et al.*, 1980a; Morton *et al.*, 1982) and in normal humans (Hollenberg, Meggs, Williams, Katz, Garnic and Harrington, 1981; MacGregor, Markandu, Roulston and Jones, 1981). The two latter studies also demonstrated an increase in sodium excretion associated with the captopril administration. As might be expected, in all cases the drop in systemic blood pressure following captopril administration in the salt replete state was smaller than that caused by captopril during salt depletion or high renin hypertension. The increase in renal plasma flow caused by captopril in high and low renin states did not show such a marked difference. The results of these studies demonstrating the effects of captopril in the normotensive salt replete state have been taken to indicate a tonic influence of the renin-angiotensin system on systemic blood pressure, renal plasma flow and sodium excretion under normal conditions.

The ability of captopril to affect systemic and renal haemodynamics during sodium repletion could, however, also indicate that captopril may be mediating its effect through mechanisms independent of inhibition of the renin-angiotensin system, and indeed much controversy exists as to the specificity of action of captopril.

Some investigators have tried to restore blood pressure during captopril administration by infusing angiotensin II. On an acute basis, infusion of angiotensin II into captopril-treated salt replete and salt deplete conscious dogs failed to restore systemic blood pressure to pre-captopril levels (Tree and Morton, 1980). Captopril administration in the salt deplete dogs also produced a shift to the right and a marked downward shift in the angiotensin II/blood pressure dose-response curve. This implied that diminution in the acute vasoconstrictor effect of angiotensin II consequent to a reduction in its plasma concentration is not the sole mechanism by which captopril lowered systemic blood pressure. Chronic infusion of angiotensin II into captopril-treated salt-deplete conscious dogs restored systemic blood pressure and renal plasma flow to control levels (Hall *et al.*, 1979b), although high doses of angiotensin II were used and lower dose chronic angiotensin II infusion failed to return systemic blood pressure to control values. It would seem, therefore, that a mechanism additional to inhibition of angiotensin II formation may be mediating the hypotensive action of captopril. This hypothesis was supported by investigations into the effects of captopril in low-renin hypertensive states. Captopril was found to be effective in lowering systemic blood pressure in patients with low-renin essential hypertension (Gavras *et al.*, 1978; Abe, Itoh, Imai, Satoh Haruyama, Sakurai Goto, Otsuka and Yoshinaga, 1980) and in spontaneously hyper-

tensive rats (Muirhead, Prewitt, Brooks and Brosius, 1978), angiotensin-salt hypertensive rats (Muirhead, Brooks and Brosius, 1980), and DOCA/salt hypertensive rats (Miyamori, Brown and Dollery, 1980) in all of which plasma renin activity is known to be low.

By inhibiting angiotensin I converting enzyme, also known as kininase II, captopril not only blocks the conversion of angiotensin I to angiotensin II, but prevents the degradation of bradykinin (Erdös, 1977). Captopril may therefore cause increased circulating and renal tissue concentrations of kinins. Bradykinin is a potent vasodilator and infusion of bradykinin into the renal artery of dogs causes an increase in renal blood flow, urine flow and sodium excretion (Barraclough and Mills, 1965; Stein, Congbalay, Karsh, Osgood and Ferris, 1972; Nasjletti, Colina-Churito and McGiff, 1975). Potentiation of these effects due to inhibition of the degradation of bradykinin may contribute towards the effects of captopril administration on renal haemodynamics. Direct evidence for a contribution of increased bradykinin levels towards the hypotensive or renal vasodilator effect of captopril, however, remains conflicting.

Increased plasma kinin levels after captopril administration have been reported in conscious rats (Matthews and Johnston, 1980) and conscious salt-deplete dogs (McCaa *et al.*, 1978), whilst other investigators have failed to demonstrate any effect of captopril on plasma kinin levels in hypertensive patients (Johnston, Millar, McGrath and Matthews, 1979; Crantz, Swartz, Hollenberg, Moore, Dluhy and Williams, 1981). The renal kallikrein-kinin system is regarded as a local hormonal system which by regulation of regional blood flow may be important in circulatory homeostasis and blood pressure control (Levinsky, 1979; Carretero

and Scicli, 1980). Urinary kinins are thought to originate only from renal tissue and may parallel intrarenal concentrations of these substances (Yoshinaga, Abe, Miwa, Funujama and Suzuki, 1964; Orstavik, Nustad, Brandtzaeg and Pierce, 1976). Urinary kinin levels have been found to increase after captopril administration in conscious salt-deplete dogs (Olsen & Arrigoni-Martelli 1979). In anaesthetised dogs, captopril administration resulted in increased urinary kinin levels while plasma levels did not change (Johnston, Clappison, Anderson and Yasiyima, 1982). These results indicate that whilst an increase in renal bradykinin may participate in the renal vasodilator action of captopril, a contribution of circulating bradykinin to the hypotensive action of captopril seems unlikely.

The vasodilator action of bradykinin is thought to be mediated in part by a prostaglandin (McGiff *et al.*, 1973; Mullane and Moncada, 1980) and it has been demonstrated that bradykinin can stimulate PGE_2 and PGI_2 synthesis from a variety of vascular beds (Piper and Vane, 1969; Needleman *et al.*, 1975; Blumberg *et al.*, 1977; Hong, 1980). Bradykinin has also been shown to stimulate PGE_2 and PGI_2 synthesis in the kidney (McGiff *et al.*, 1973; Blasingham and Nasjletti, 1979; Mullane and Moncada, 1980). Since bradykinin stimulates prostaglandin synthesis, it has been proposed that captopril administration, by elevating circulating or tissue kinins, may increase PGE_2 and PGI_2 levels. These prostaglandins, by means of their vasodilator and natriuretic properties, may consequently contribute to the actions of captopril. Alternatively, since angiotensin II also promotes PGE_2 and PGI_2 synthesis in a number of different tissues (McGiff *et al.*, 1970b; Shebuski and Aiken, 1980; Dusting and Mullins, 1980), captopril may reduce prostaglandin synthesis by lowering angiotensin II levels.

Several studies have investigated the contribution of prostaglandins to the hypotensive and renal vasodilator actions of captopril and the results have proved conflicting. Indomethacin has been shown to inhibit an enhanced vasodepressor effect of bradykinin following captopril administration in conscious rabbits, although this inhibition could not be repeated in nephrectomised animals (Murthy, Waldron and Goldberg, 1978). An enhanced hypotensive and renal vasodilator response to bradykinin following captopril administration in anaesthetised dogs was associated with an increased release of PGI_2-like material primarily from the renal circulation and was inhibited by indomethacin (Mullane and Moncada, 1980). In anaesthetised rats the vasodepressor response to bradykinin was potentiated following captopril administration but indomethacin was unable to inhibit this effect (Vandongen, Tunney, Barden and Mahoney, 1982). This suggested that the potentiation of the vasodilator action of bradykinin was not mediated by a prostaglandin.

Similarly conflicting results have been obtained when the effect of indomethacin on the hypotensive response to captopril has been studied. Indomethacin pretreatment attenuated the hypotensive action of captopril in DOCA/salt hypertensive rats but had no effect on the captopril-induced increase in plasma renin activity (Miyamori *et al.*, 1980). Indomethacin also attenuated the fall in systemic blood pressure after captopril administration in patients with reno-vascular hypertension but again had no effect on the increase in plasma renin activity (Salvetti, Arzilli, Pedrinelli and Beggi, 1980). However, other investigators have demonstrated an attenuation by indomethacin of both the hypotension and increased plasma renin activity due to captopril administration in normotensive and hypertensive patients (Abe, Itoh, Satoh, Haruyama, Imai, Goto, Satoh, Otsuka and Yoshinaga, 1979; Silberbauer, Stanek,

and Templ , 1982; Witzgall, Scherer and Weber, 1982). Conversely, both indomethacin and aspirin treatment failed to modify the hypertensive action of chronic captopril administration in spontaneously hypertensive rats (Antonacio, Harris, Goldenberg, High and Rubin, 1979; DiNicolantonio, Dusting, Hutchison and Mendelsohn, 1981) and no attenuation by indomethacin of the hypotensive or renal vasodilator action of captopril could be demonstrated in anaesthetised or conscious dogs (Tobia and Giardino, 1981; Wong, Zimmerman, Kraft, Kounenis and Friedman, 1981).

The depressor response to captopril has been positively correlated to the change in 13,14-dihydro-15-keto-PGE_2 in the plasma of sodium replete and deplete humans (Swartz, Williams, Hollenberg, Levine, Dluhy and Moore, 1980) and in patients with essential hypertension (Moore, Crantz, Hollenberg, Kotesky, Leboff, Swartz, Levine, Podolsky, Dluhy and Williams, 1981). No changes in plasma 6-keto-$PGF_{1\alpha}$ or TxB_2 levels were detected, although this may be due to the difficulty in measuring the small quantities of these metabolites in the plasma. Other investigators have failed to demonstrate an effect of captopril on renal venous PGE_2 levels in haemorrhaged anaesthetised dogs (Wong and Zimmerman, 1980b) or on urinary PGE_2 excretion in conscious dogs (Olsen and Arrigoni-Martelli, 1979). No increase in urinary PGE_2 excretion following captopril administration could be demonstrated in normotensive or hypertensive humans, despite an attenuation of the vasodepressor response by indomethacin (Abe *et al.*, 1979; Witzgall *et al.*, 1982). These '*in vivo*' studies looked at the effects of captopril on PGE_2 synthesis only and it is possible that PGI_2, which is a more potent renin secretagogue (Jones Watson & Ungar, 1981), may be a more important mediator of the effects of captopril.

Some evidence exists towards a direct action of captopril to increase prostaglandin synthesis. Galler *et al*. demonstrated an increase in both PGE_2 and PGI_2 synthesis by isolated rat glomeruli incubated with captopril but could not demonstrate a similar effect using aortic strips (Galler, Backenroth, Folkert and Schlondorff, 1982). In contrast to this, Vio *et al*. observed an increase in PGI_2 synthesis by rat aortic strips incubated with captopril but did not observe any increase in PGI_2 synthesis when renal medulla was incubated with captopril (Vio, Guivernau, Terragno and Terragno, 1981).

In the first study in this section, captopril has been used as a pharmacological tool to investigate the relative importance of the renin-angiotensin system in the control of systemic and renal haemodynamics and electrolyte excretion under conditions of differing sodium balance. Studies were performed in conscious dogs in both sodium replete and sodium deplete states. In addition, to determine whether PGI_2 contributes in any way to the haemodynamic and/or renin secretory actions of captopril, urinary 6-keto-$PGF_{1\alpha}$ excretion has been measured before and after captopril administration.

The second study in this section has further utilised captopril to investigate the complex inter-relationships between the renin-angiotensin system and PGI_2. It is generally thought that the vasodilator action of PGI_2 plays an important role in modulating the vasoconstrictor action of angiotensin II, particularly in the kidney. Indomethacin enhances the renal vasoconstrictor and vasopressor action of exogenous angiotensin II (Aiken and Vane, 1973; Negus *et al*., 1976) and causes an increase in renal vascular resistance which is much more pronounced during sodium depletion (Blasingham and Nasjletti, 1980). It is possible that angiotensin II stimulates PGI_2 synthesis in the kidney which then

serves to modulate its vasoconstrictor activity. It is also possible that angiotensin II may be modulating the vasodilator action of PGI_2 since PGI_2 is capable of stimulating renin release with a consequent increase in angiotensin II formation.

In the second study, PGI_2 was infused intravenously into conscious dogs after angiotensin II formation was inhibited by captopril. The vasodilator action of PGI_2 after inhibition of angiotensin II formation could then be compared to the expected action were angiotensin II present. The PGI_2 infusions were performed in conscious sodium replete and deplete dogs. The effects of an alteration in the control of systemic and renal haemodynamics by the renin-angiotensin system on the vasodilator action of PGI_2 could then be determined.

4.2 Study I Methods

Eight male conscious foxhounds were used, all dogs being surgically prepared as described in section 2.1. The animals were maintained on diets containing either 50 mMoles sodium/day or less than 10 mMoles sodium/day (detailed in section 2.3).

On the day of each study, dextrose solution (5 gm/100 ml) was infused intravenously at 7 ml/min for 30 min, then continued at 3 ml/min throughout the rest of the study. This established a modest water diuresis. Loading concentrations of PAH and inulin were administered and the appropriate dilutions infused intravenously at 0.5 ml/min. A 19 G butterfly needle was inserted into the carotid artery and connected to a pressure transducer to allow continuous measurement of systemic blood pressure. A one-hour equilibration period was allowed before the 4-hour study commenced. After a 90 min control period, captopril (5 mg/

kg) dissolved in a small quantity of saline was administered intravenously as a bolus injection and its effects monitored for a further 150 min. *

Throughout the 4 hour study, urine collections were taken every 30 min via a catheter inserted into the bladder. Arterial blood samples were taken during the control period and 30, 60 and 150 min after captopril administration. Systemic blood pressure, renal plasma flow, glomerular filtration rate, sodium, potassium and osmolality excretion, plasma renin activity and urinary 6-keto-$PGF_{1\alpha}$ excretion were estimated by techniques described fully in sections 2.5 - 2.6 . At least 48 hours were allowed between each study.

4.3 Statistical Comparisons

All results are expressed as mean ± standard error. Statistical comparisons for all parameters except 6-keto-$PGF_{1\alpha}$ excretion and plasma renin activity were made using a student 2-tailed t-test between the points after captopril administration and the control point immediately preceding captopril administration. Although 6-keto-$PGF_{1\alpha}$ excretion rates and plasma renin activity are graphically represented as mean ± standard error, they were not thought to be normally distributed. Therefore statistical comparisons were made using the non-parametric Wilcoxan's rank sum test for paired data. Again the points after captopril administration were compared with the control point immediately preceding captopril administration.

Significance level for both tests was taken at $p < 0.05$.

* see footnote

4.4 Results

The effects of captopril administration on systemic blood pressure, renal haemodynamics, electrolyte excretion, urinary 6-keto-$PGF_{1\alpha}$ excretion and plasma renin activity in sodium replete and sodium deplete conscious dogs are summarised in Tables 4.1 - 4.3.

4.4.1 Systemic blood pressure (Fig. 4.1)

Systemic blood pressure was slightly lower during sodium depletion than when animals were maintained on a normal sodium intake. In the sodium replete animals, captopril caused systemic blood pressure to fall from 97 ± 3 mmHg to 90 ± 3 mmHg within 30 min of administration. This decrease, though small, was statistically significant ($p < 0.01$). Systemic blood pressure showed a small upward trend towards the end of the study. There was a greater fall in systemic blood pressure in the sodium deplete animals where pressure decreased from 93 ± 2 mmHg to 74 ± 2 mmHg within 30 min of captopril administration. This decrease was highly significant ($p < 0.001$), however there was an upward trend in the pressure towards the end of the 150 min period.

4.4.2 Heart rate (Fig. 4.1)

Heart rate was not altered by sodium deprivation. Captopril administration increased heart rate from 77 ± 7 beats/min to 100 ± 7 beats/min ($p < 0.01$) in the salt replete dogs and from 76 ± 4 beats/min to 106 ± 9 beats/min ($p < 0.01$) in the salt deplete. In both cases, the maximum increase occurred within 30 min of captopril administration after which heart rate started to decrease towards control values. Captopril increased heart rate to the same extent in both the salt replete and salt deplete states.

TABLE 4.1:

Captopril administration ↓

Parameter	-60M	-30M	0M	30M	60M	90M	120M	150M
Systemic blood pressure (mm Hg)								
high sodium	97 ± 3	96 ± 5	97 ± 3	90 ± 3	90 ± 3	93 ± 4	92 ± 4	94 ± 4
low sodium	91 ± 2	92 ± 2	93 ± 2	74 ± 2	73 ± 2	75 ± 3	76 ± 2	78 ± 4
Heart rate (beats/min)								
high sodium	80 ± 9	80 ± 5	77 ± 7	100 ± 7	94 ± 6	96 ± 11	90 ± 5	87 ± 8
low sodium	86 ± 6	74 ± 6	76 ± 4	106 ± 9	100 ± 6	95 ± 7	91 ± 6	99 ± 8
Renal plasma flow (ml/min)								
high sodium	312 ± 66	329 ± 64	310 ± 71	347 ± 50	367 ± 74	378 ± 60	293 ± 41	310 ± 33
low sodium	355 ± 45	275 ± 39	350 ± 47	309 ± 19	451 ± 73	463 ± 64	371 ± 50	352 ± 34
Glomerular filtration rate (ml/min)								
high sodium	95 ± 12	93 ± 13	84 ± 13	99 ± 12	87 ± 10	103 ± 11	81 ± 10	89 ± 12
low sodium	89 ± 9	88 ± 11	88 ± 8	59 ± 9	89 ± 4	95 ± 10	93 ± 11	93 ± 7
Filtration fraction								
high sodium	0.31 ± 0.03	0.28 ± 0.05	0.27 ± 0.04	0.28 ± 0.03	0.23 ± 0.02	0.26 ± 0.05	0.27 ± 0.02	0.28 ± 0.02
low sodium	0.26 ± 0.03	0.32 ± 0.02	0.26 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	0.25 ± 0.02	0.26 ± 0.02

n = 8

TABLE 4.2:

Captopril administration
↓

Parameter	-60M	-30M	0M	30M	60M	90M	120M	150M
Sodium excretion (μM/min)								
high sodium	43 ± 9	40 ± 15	29 ± 10	79 ± 21	92 ± 22	116 ± 26	112 ± 31	133 ± 22
low sodium	1.9 ± 0.7	1.4 ± 0.4	1.2 ± 0.6	2.1 ± 0.9	3.2 ± 1.0	2.7 ± 0.9	4.1 ± 1.2	3.3 ± 1.3
Potassium excretion (μM/min)								
high sodium	34 ± 5	36 ± 6	32 ± 6	47 ± 4	42 ± 8	57 ± 13	40 ± 10	43 ± 13
low sodium	38 ± 6	36 ± 5	35 ± 5	30 ± 5	42 ± 4	59 ± 12	56 ± 14	48 ± 8
Urine flow (ml/min)								
high sodium	3.1 ± 0.4	3.9 ± 0.5	3.2 ± 0.6	4.4 ± 0.5	3.8 ± 0.4	4.7 ± 0.4	3.3 ± 0.4	2.7 ± 0.5
low sodium	3.9 ± 0.4	3.3 ± 0.3	3.3 ± 0.4	2.5 ± 0.5	2.9 ± 0.5	3.8 ± 0.8	3.5 ± 0.1	3.1 ± 0.6
Osmolality excretion (μ Osm/min)								
high sodium	529 ± 72	586 ± 88	441 ± 46	568 ± 66	592 ± 78	721 ± 67	561 ± 85	597 ± 66
low sodium	640 ± 57	521 ± 53	534 ± 90	374 ± 76	488 ± 47	520 ± 65	541 ± 71	455 ± 49
Free water clearance (ml/min)								
high sodium	1.8 ± 0.4	1.9 ± 0.4	2.1 ± 0.5	2.5 ± 0.3	1.9 ± 0.4	2.3 ± 0.3	1.4 ± 0.2	1.3 ± 0.3
low sodium	1.3 ± 0.4	1.6 ± 0.3	1.7 ± 0.3	1.1 ± 0.2	1.4 ± 0.3	2.2 ± 0.5	2.0 ± 0.4	1.7 ± 0.4

n = 8

TABLE 4.3:

Captopril administration ↓

Parameter	-60M	-30M	0M	30M	60M	90M	120M	150M
6-keto-$PGF_{1\alpha}$ excretion (ng/min)								
high sodium	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	0.9 ± 0.2	1.2 ± 0.3	2.1 ± 0.4	1.3 ± 0.3	1.3 ± 0.5
low sodium	1.1 ± 0.4	1.2 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.9 ± 0.7	2.4 ± 0.9	1.7 ± 0.5	1.8 ± 0.7
Plasma renin activity (ng/ml/hr)								
high sodium	1.7 ± 0.5		2.2 ± 0.4	1.4 ± 0.2		2.9 ± 0.9		4.3 ± 1.0
low sodium	8.5 ± 1.2		8.8 ± 1.3	49.8 ± 19.0		36.0 ± 11.9		26.4 ± 8.1

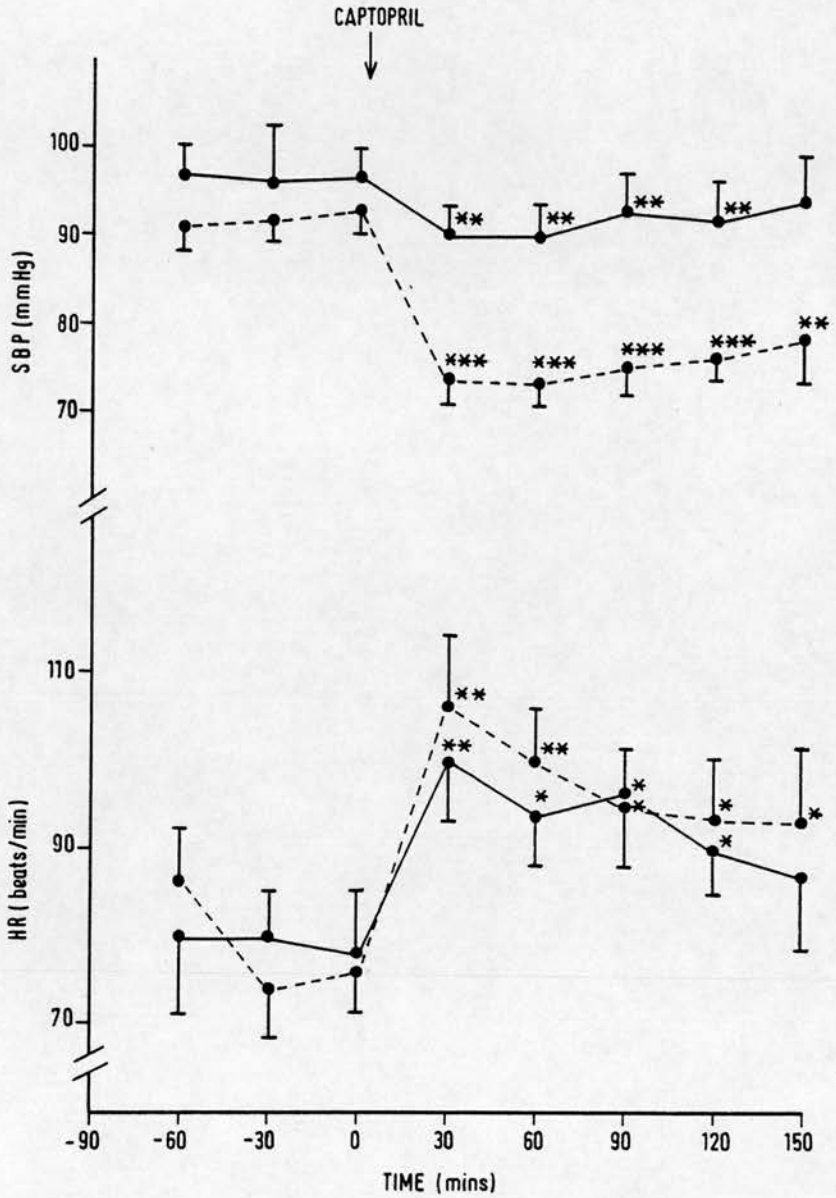


FIGURE 4.1: The effect of captopril administration on systemic blood pressure and heart rate.

sodium replete dogs;
sodium deplete dogs.

* p <0.05; ** p <0.01; *** p <0.001.

4.4.3 Renal plasma flow (Fig. 4.2)

Sodium depletion had no effect on the control values for renal plasma flow. In both sodium replete and deplete dogs, captopril caused an increase in renal plasma flow which was maximal 90 min after administration. In the salt replete dogs, renal plasma flow was increased by 68 ± 10 ml/min, from a control value of 310 ± 71 ml/min to 378 ± 60 ml/min 90 min after captopril administration ($p < 0.05$). Captopril had a greater effect on renal plasma flow in salt deplete dogs where an increase of 113 ± 16 ml/min was observed, from a control value of 350 ± 47 ml/min to 463 ± 64 ml/min 90 min after captopril administration ($p < 0.05$). In both salt replete and salt deplete dogs, renal plasma flow started to decrease towards control levels by 120 min after captopril administration.

4.4.4 Glomerular filtration rate (Fig. 4.2)

Glomerular filtration rate was not altered by sodium depletion. Captopril administration had no effect on glomerular filtration rate in the salt replete dogs. There was a transient decrease in glomerular filtration rate in the salt deplete dogs 30 min after captopril administration. This transient fall in glomerular filtration rate most likely reflects the large fall in systemic blood pressure seen at this time.

4.4.5 Filtration fraction (Fig. 4.2)

Filtration fraction showed very little change after captopril administration in the salt replete dogs. There was a small decrease 60 min after captopril administration but this was not statistically significant. Captopril caused a larger decrease in filtration fraction in the salt deplete dogs, from 0.26 ± 0.02 to 0.19 ± 0.02 within 30 min of administration ($p < 0.001$). The decrease in filtration fraction in the salt deplete

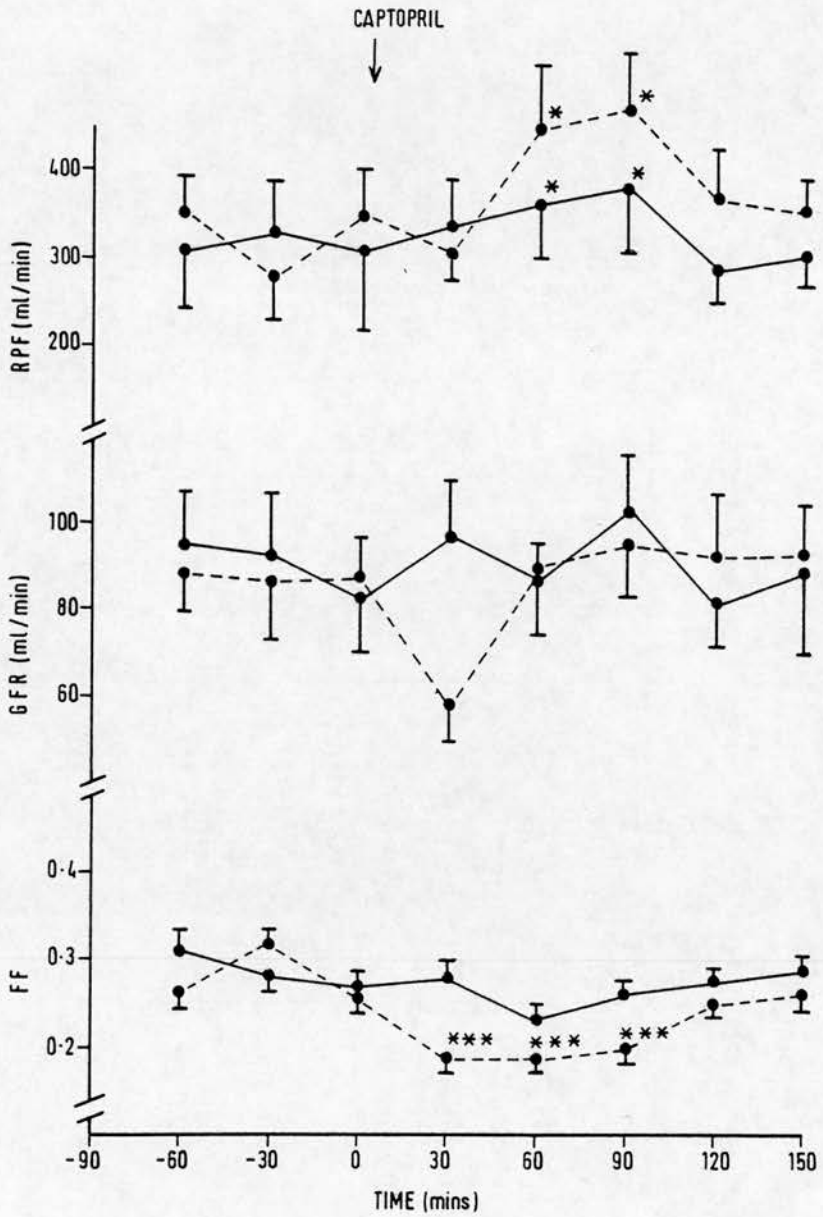


FIGURE 4.2: The effect of captopril administration on renal plasma flow, glomerular filtration rate and filtration fraction.

sodium replete dogs;

sodium deplete dogs.

* p <0.05; ** p <0.01; *** p <0.001.

dogs is due initially to the reduced glomerular filtration rate 30 min after captopril administration and then, although glomerular filtration rate rises again, renal plasma flow also rises resulting in a maintained reduction in filtration fraction. Filtration fraction in the salt deplete dogs increases towards the end of the study when renal plasma flow decreases.

4.4.6 Sodium excretion (Fig. 4.3)

Sodium excretion rates during the control periods were much smaller during sodium deprivation than when animals were maintained on a normal sodium intake. Captopril caused a large increase in sodium excretion in the salt replete dogs within 30 min of administration and sodium excretion continued to increase throughout the study. By 150 min after captopril administration, sodium excretion was increased from a control value of 29 ± 10 μM/min to 133 ± 22 μM/min ($p < 0.01$). Captopril also increased sodium excretion in the salt deplete dogs within 30 min of administration and although sodium excretion remained elevated, it tended to fluctuate throughout the study. Sodium excretion was increased from a control value of 1.2 ± 0.6 μM/min to 3.3 ± 1.3 μM/min 150 min after captopril administration in the salt deplete dogs ($p < 0.05$).

4.4.7 Potassium excretion (Fig. 4.3)

Potassium excretion during the control periods was not altered by sodium deprivation. Captopril caused an increase in potassium excretion in both salt replete and salt deplete dogs, the maximum effect being 90 min after administration. In the salt replete dogs, potassium excretion increased from a control of 32 ± 6 μM/min to 57 ± 13 μM/min 90 min after captopril administration ($p < 0.05$). A similar increase was

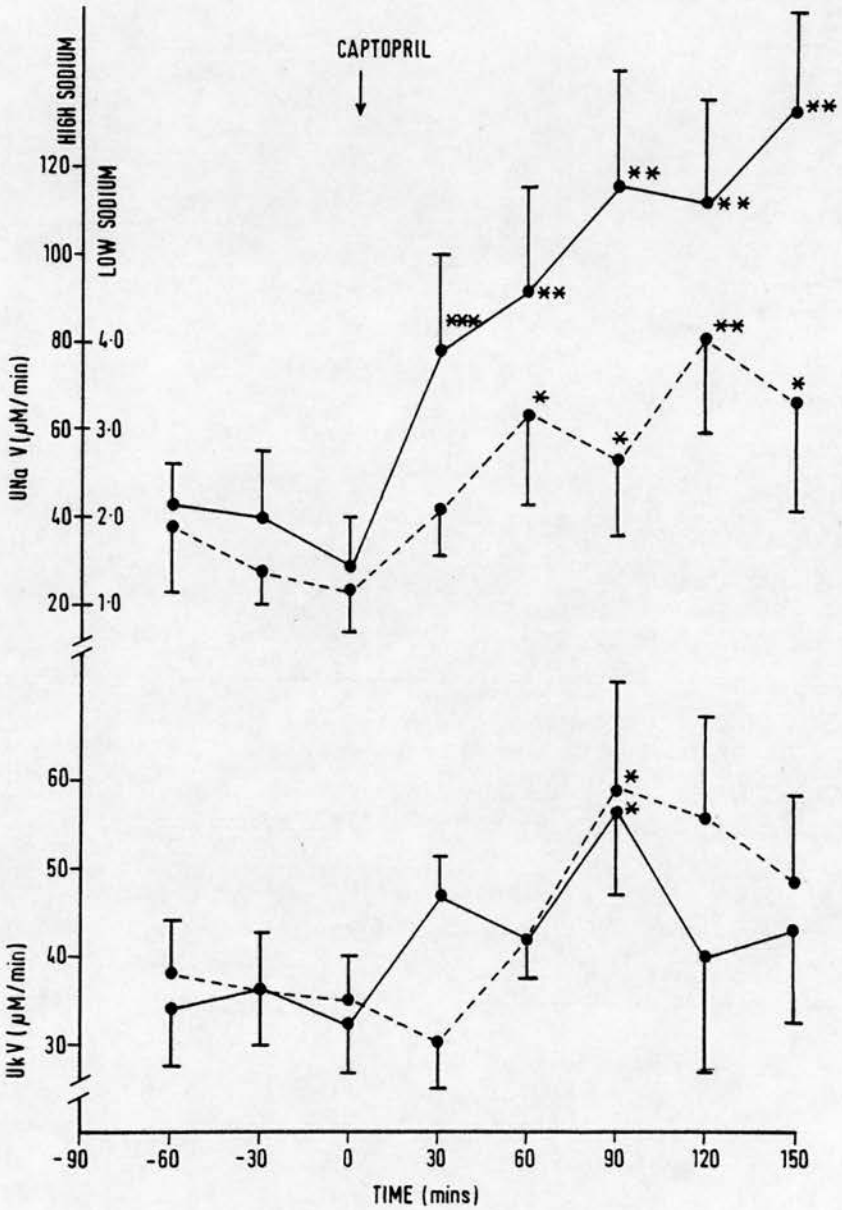


FIGURE 4.3: The effect of captopril administration on sodium and potassium excretion.

●——● sodium replete dogs;

●- - -● sodium deplete dogs.

* p <0.05; ** p <0.01; *** p <0.001.

demonstrated in the salt deplete dogs where potassium excretion increased from a control value of 35 ± 5 μM/min to 59 ± 12 μM/min 90 min after captopril administration ($p < 0.05$). The increase in potassium excretion following captopril administration follows a similar time course to the changes in renal plasma flow, both parameters demonstrating a maximum increase 90 min after captopril administration.

4.4.8 Urine flow (Fig. 4.4)

Urine flow showed a small increase after captopril administration in the salt replete dogs but fell to control levels again by the end of the study. There was a small decrease in urine flow after captopril administration in the salt deplete dogs. This most likely reflects the initial decrease in glomerular filtration rate observed after captopril administration in the salt deplete dogs.

4.4.9 Solute (osmolality) excretion (Fig 4.4)

Solute excretion was not altered by sodium depletion. There was an increase in solute excretion after captopril administration in the salt replete dogs, probably as a consequence of the increase in sodium excretion. There was no increase in osmolality excretion after captopril administration in the salt deplete dogs.

4.4.10 Free water clearance (Fig. 4.4)

Free water clearance during the control periods was lower during sodium deprivation than when animals were maintained on a normal sodium intake. Free water clearance fluctuated throughout the studies and there was no significant change after captopril administration in either the salt replete or salt deplete dogs.

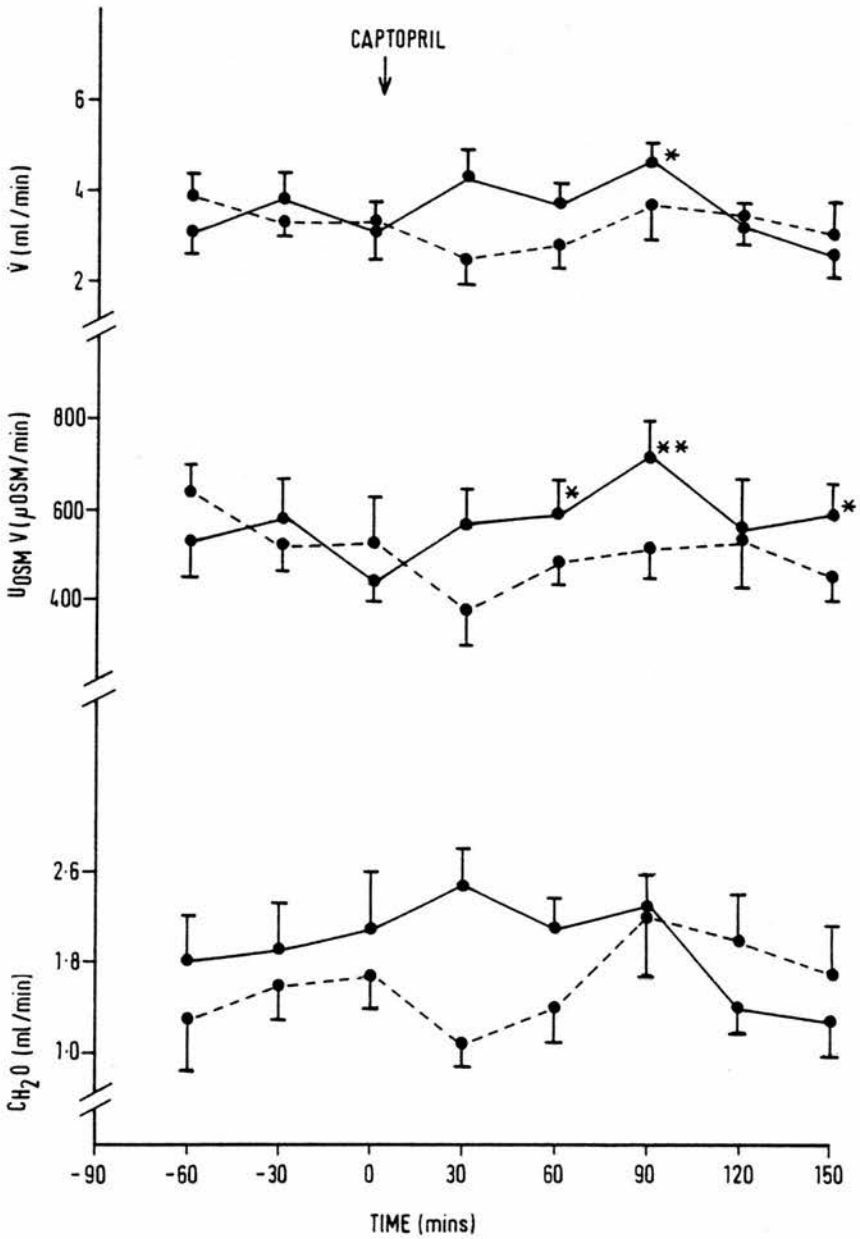


FIGURE 4.4: The effect of captopril administration on urine flow, osmolality excretion and free water clearance.

●——● sodium replete dogs;

●- - - -● sodium deplete dogs.

* p <0.05; ** p <0.01; *** p <0.001.

4.4.11 6-keto-$PGF_{1\alpha}$ excretion (Fig. 4.5)

Urinary 6-keto-$PGF_{1\alpha}$ excretion during the control periods was not different during sodium deprivation than when animals were maintained on a normal sodium intake. Captopril caused an increase in 6-keto-$PGF_{1\alpha}$ excretion which was maximal 90 min after administration in both salt replete and salt deplete dogs. In the salt replete dogs 6-keto-$PGF_{1\alpha}$ excretion increased from a control value of 1.2 ± 0.2 ng/min to 2.1 ± 0.4 ng/min 90 min after captopril administration ($p < 0.02$). A similar increase in 6-keto-$PGF_{1\alpha}$ excretion was demonstrated in salt deplete dogs where 6-keto-$PGF_{1\alpha}$ excretion increased from a control value of 1.3 ± 0.3 ng/min to 2.4 ± 0.9 ng/min 90 min after captopril administration ($p < 0.05$). The mean urinary 6-keto-$PGF_{1\alpha}$ excretion rates could be equated with circulating PGI_2 levels as defined by the relationship between PGI_2 infusion rate and urinary 6-keto-$PGF_{1\alpha}$ excretion demonstrated by Figure 3.9 in section 3.4.12. By this means, the calculated rate of entry of PGI_2 into the circulation 90 min after captopril administration was 3.6 ng/kg/min in the salt replete dogs and 4.3 ng/kg/min in the salt deplete dogs.

The time-course for the increase in 6-keto-$PGF_{1\alpha}$ excretion resembles that for the increase in renal plasma flow, both parameters reaching a maximum 90 min after captopril administration.

4.4.12 Plasma renin activity (Fig. 4.6)

Plasma renin activity was greatly increased by sodium deprivation demonstrating the stimulation of the renin-angiotensin system. Plasma renin activity showed a small progressive increase after captopril administration in the salt replete dogs. However, this increase from a control value of 1.2 ± 0.2 ng/ml/hr to 4.3 ± 1.0 ng/ml/hr 150 min after captopril

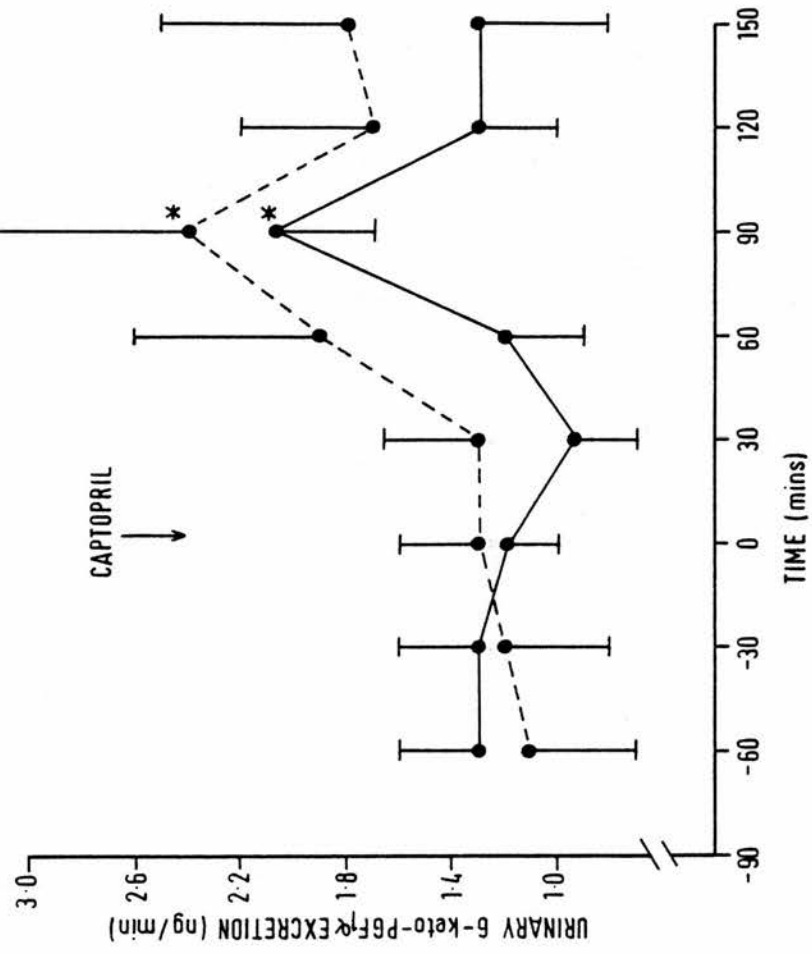


FIGURE 4.5: The effect of captopril administration on 6-keto-$PGF_{1\alpha}$ excretion.

● sodium replete dogs; ● - - - ● sodium deplete dogs.

* $p < 0.05$.

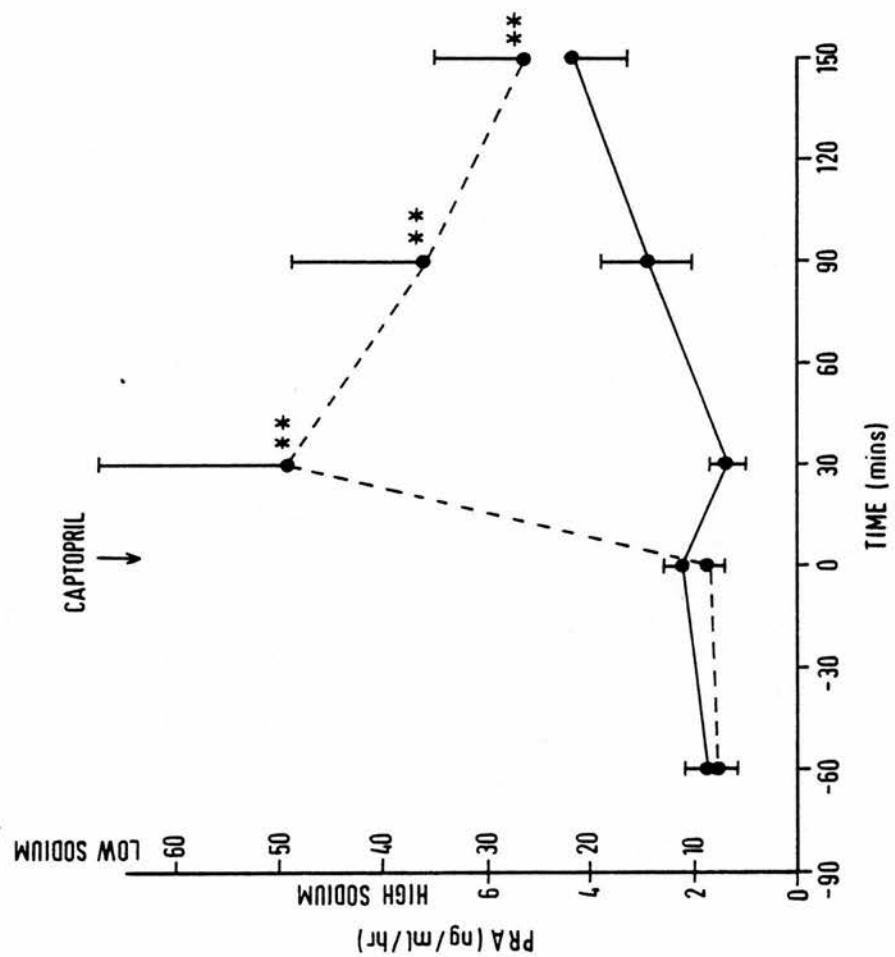


FIGURE 4.6: The effect of captopril administration on plasma renin activity.

● sodium replete dogs; ●– – –● sodium deplete dogs.

** p <0.01.

administration did not reach statistical significance. In the salt deplete dogs, captopril administration resulted in an increase in plasma renin activity from a control value of 8.8 ± 1.3 ng/ml/hr to 49.8 ± 19 ng/ml/hr within 30 min of administration ($p < 0.01$). Plasma renin activity then fell to 26 ± 8.1 ng/ml/hr by 150 min after captopril administration ($p < 0.01$).

4.5 Study II Methods

Eight male conscious foxhounds were used, all animals being surgically prepared as described in section 2.1. The dogs were maintained on diets containing either 50 mM sodium/day or less than 10 mM sodium/day as detailed in Section 2.3.

On the day of each study, dextrose solution (5 gm/100 ml) was infused intravenously at 7 ml/min for 30 min, then continued at 3 ml/min throughout the rest of the study. This established a modest water diuresis. Loading concentrations of PAH and inulin were administered, and the appropriate dilutions infused intravenously at 0.5 ml/min. A 19G butterfly needle inserted into the carotid artery and connected to a pressure transducer allowed continuous measurement of systemic blood pressure. A one hour equilibration period was allowed before the four hour study was commenced. After a 90 min control period, captopril (5 mg/kg) was administered intravenously as a bolus injection. 30 min after the captopril administration, PGI_2 in a concentration of 15 ng/kg/min was infused intravenously at a rate of 0.5 ml/min. This infusion was continued for 120 min. The PGI_2 was dissolved in 0.025M sodium carbonate buffer (pH 9.8) during the sodium replete study, and 0.025M potassium carbonate buffer (pH 9.8) during the sodium deplete study.

The infusion solution was replaced every 30 min by a fresh solution made up from stock PGI_2 kept on ice. The activity of the PGI_2 in the infusion solution was checked on several occasions by its ability to prevent ADP-induced platelet aggregation. Throughout the 4 hour study, urine collections were made every 30 min via a catheter inserted into the bladder. Arterial blood samples were taken during the control period, 30 min after captopril administration and 60 and 120 min after the start of the PGI_2 infusion.

Systemic blood pressure, renal plasma flow, glomerular filtration rate, sodium, potassium and osmolality excretion and plasma renin activity were estimated by techniques described fully in Section 2.5.

At least 48 hours were allowed between each study.

4.6 Statistical Comparisons

All results are expressed as mean ± standard error. Statistical comparisons were not thought to be useful in this study since the results involved the combined effects of two different drugs.

4.7 Results

The effects of captopril administration followed by infusion of PGI_2 on systemic blood pressure, renal haemodynamics, electrolyte excretion and plasma renin activity in salt replete and salt deplete dogs, are summarised in Tables 4.5 and 4.6.

4.7.1 Systemic blood pressure (Fig. 4.7)

As demonstrated in section 4.4.1, systemic blood pressure during the control periods was lower during sodium deprivation than when

TABLE 4.4

Parameter	-60M	-30M	0M	Captopril administration ↓	120M	PGI$_2$ Infusion 150M	180M	210M	240M
Systemic blood pressure (mm Hg)				c		PGI$_2$			
high sodium	93.1 ± 3.6	95.5 ± 3.5	97.0 ± 2.9		90.8 ± 3.9	85.7 ± 3.9	83.7 ± 3.9	88.0 ± 3.3	90.1 ± 3.4
low sodium	87.2 ± 1.8	88.4 ± 1.5	89.5 ± 1.6		73.2 ± 2.0	69.4 ± 2.8	71.6 ± 2.8	76.4 ± 3.0	77.8 ± 2.9
Heart rate (beats/min)				c		PGI$_2$			
high sodium	81 ± 5	84 ± 11	86 ± 10		87 ± 10	96 ± 7	97 ± 8	90 ± 7	101 ± 7
low sodium	80 ± 6	82 ± 7	86 ± 10		100 ± 8	96 ± 10	101 ± 9	105 ± 8	109 ± 7
Renal plasma flow (ml/min)				c		PGI$_2$			
high sodium	315 ± 22	325 ± 48	320 ± 40		363 ± 38	370 ± 54	419 ± 72	470 ± 53	501 ± 57
low sodium	283 ± 26	288 ± 50	289 ± 34		395 ± 60	365 ± 68	367 ± 63	412 ± 64	403 ± 59
Glomerular filtration rate (ml/min)				c		PGI$_2$			
high sodium	90 ± 7	85 ± 11	92 ± 10		83 ± 6	79 ± 11	84 ± 12	100 ± 9	115 ± 12
low sodium	88 ± 5	89 ± 11	75 ± 6		87 ± 8	81 ± 10	74 ± 8	84 ± 9	90 ± 7
Filtration fraction				c		PGI$_2$			
high sodium	0.29 ± 0.02	0.27 ± 0.02	0.28 ± 0.02		0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.02
low sodium	0.31 ± 0.03	0.31 ± 0.02	0.25 ± 0.02		0.22 ± 0.02	0.22 ± 0.02	0.20 ± 0.03	0.20 ± 0.02	0.22 ± 0.02

n = 8

TABLE 4.5:

Parameter	-60M	-30M	0M	Captopril administration ↓	120M	PGI_2 Infusion 150M	180M	210M	240M
Sodium excretion (μM/min)				c		PGI_2			
high sodium	62 ± 23	59 ± 13	62 ± 22		89 ± 29	110 ± 32	123 ± 34	168 ± 25	196 ± 37
low sodium	2.1 ± 0.7	1.7 ± 0.6	2.0 ± 0.5		3.9 ± 0.4	3.4 ± 0.4	3.2 ± 0.3	3.2 ± 0.5	2.7 ± 0.6
Potassium excretion (μM/min)				c		PGI_2			
high sodium	38 ± 3	34 ± 4	37 ± 4		52 ± 8	52 ± 8	52 ± 9	56 ± 9	56 ± 9
low sodium	38 ± 3	34 ± 4	37 ± 4		53 ± 7	44 ± 5	44 ± 5	45 ± 6	44 ± 3
Urine flow (ml/min)				c		PGI_2			
high sodium	4.2 ± 0.7	4.5 ± 0.7	4.5 ± 0.6		3.5 ± 0.5	3.0 ± 0.8	2.4 ± 0.7	2.9 ± 0.7	4.2 ± 0.5
low sodium	3.4 ± 0.3	3.4 ± 0.5	3.1 ± 0.3		3.9 ± 0.4	2.8 ± 0.5	2.8 ± 0.5	2.9 ± 0.5	3.3 ± 0.4
Osmolality excretion (μOsm/min)				c		PGI_2			
high sodium	688 ± 73	577 ± 70	699 ± 98		614 ± 68	656 ± 104	650 ± 104	804 ± 45	916 ± 85
low sodium	544 ± 57	480 ± 78	442 ± 37		507 ± 33	395 ± 69	395 ± 36	410 ± 53	473 ± 32
Free water clearance (ml/min)				c		PGI_2			
high sodium	2.2 ± 0.6	2.1 ± 0.5	2.1 ± 0.3		1.9 ± 0.5	1.3 ± 0.4	1.4 ± 0.2	1.3 ± 0.3	1.6 ± 0.5
low sodium	1.7 ± 0.3	1.9 ± 0.3	1.7 ± 0.3		1.9 ± 0.4	1.7 ± 0.3	1.5 ± 0.2	1.7 ± 0.4	1.9 ± 0.5
Plasma renin activity (ng/ml/hr)				c		PGI_2			
high sodium	1.7 ± 0.3		1.9 ± 0.4		3.1 ± 0.5		5.6 ± 1.0		5.0 ± 1.0
low sodium	6.6 ± 0.9		6.9 ± 0.7		54 ± 14		49 ± 11		24 ± 5

n = 8

animals were maintained on a normal sodium intake. Captopril administration caused an immediate fall in systemic blood pressure which was greater in the salt deplete dogs. There was a further fall in systemic blood pressure after the start of the PGI_2 infusion in both salt replete and salt deplete dogs. In the salt replete dogs, systemic blood pressure fell from a value of 90.8 ± 3.9 mmHg after captopril administration to 83.7 ± 3.9 mmHg 60 min after the start of the PGI_2 infusion, a decrease of 7.1 ± 0.5 mmHg. In the salt deplete dogs, a smaller decrease in systemic blood pressure was observed following PGI_2 infusion, from a value of 73.2 ± 2 mmHg after captopril administration to 69.4 ± 2.8 mmHg 30 min after the start of the PGI_2 infusion, a decrease of 3.8 ± 0.3 mmHg. These small decreases were transient, and by the end of the study, systemic blood pressure had returned to the value reached 30 min after captopril administration, i.e. immediately prior to the start of the PGI_2 infusion.

4.7.2 Heart rate (Fig. 4.7)

Heart rate was not altered by sodium depletion confirming the results in section 4.4.2. Heart rate showed a small increase during the control periods in both the salt replete and salt deplete dogs. There was very little change in heart rate after captopril administration in the salt replete dogs and although there was an initial increase in heart rate during the PGI_2 infusion, this increase was not maintained throughout the infusion period. In the salt deplete dogs, captopril caused an increase in heart rate but there was no further increase during the PGI_2 infusion.

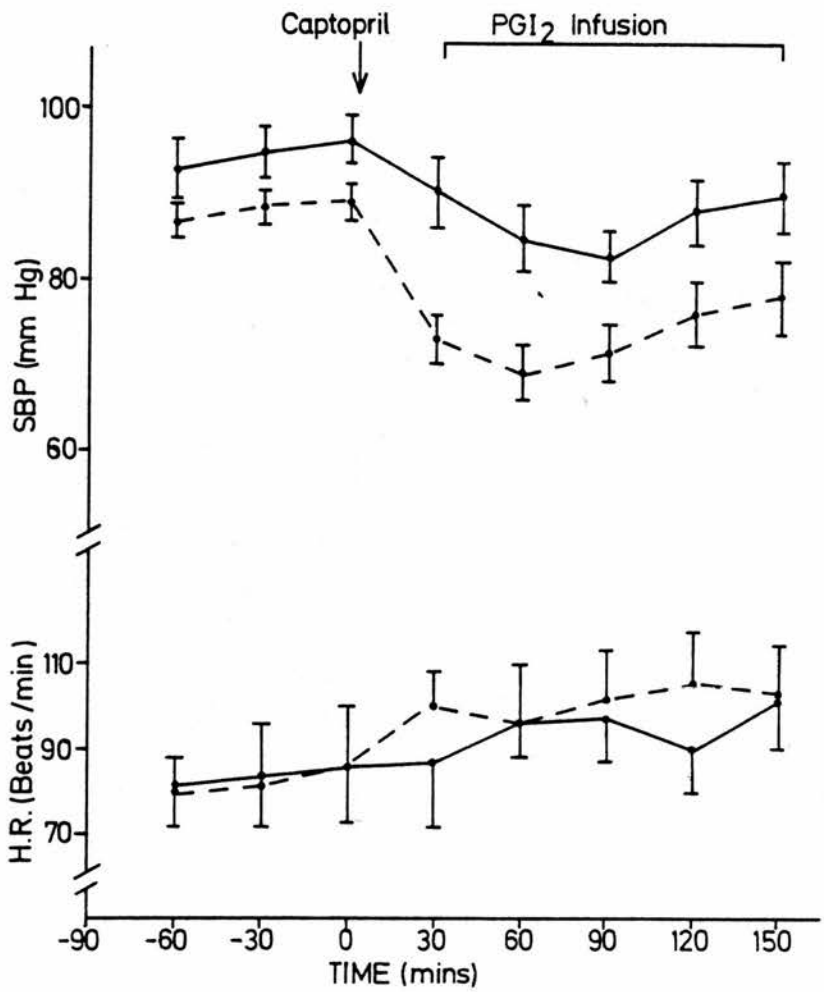


FIGURE 4.7: The effect of captopril administration and intravenous PGI_2 infusion on systemic blood pressure and heart rate.

●——● sodium replete dogs;

●– – –● sodium deplete dogs.

4.7.3 Renal plasma flow (Fig. 4.8)

Renal plasma flow during the control periods was not altered by sodium depletion confirming the results in section 4.4.3. In the salt replete dogs, renal plasma flow increased slightly from a control value of 320 ± 40 ml/min to 363 ± 38 ml/min 30 min after captopril administration, and then showed a further progressive increase during the PGI_2 infusion, reaching a flow rate of 501 ± 57 ml/min by the end of the study. Captopril administration caused a larger initial rise in renal plasma flow in the salt deplete dogs, from a control value of 289 ± 34 ml/min to 395 ± 60 ml/min 30 min after captopril administration. This increase was maintained for the duration of the study but there was no further increase in renal plasma flow during the PGI_2 infusion.

4.7.4 Glomerular filtration rate (Fig. 4.8)

Glomerular filtration rate during the control periods was not altered by sodium deprivation, confirming the results in section 4.4.4. Captopril administration had no effect on glomerular filtration rate in either the salt replete or salt deplete dogs. There was a progressive increase in glomerular filtration rate towards the end of the PGI_2 infusion in the salt replete dogs, increasing from 83 ± 6 ml/min immediately prior to the start of the infusion, to 115 ± 12 ml/min by the end of the study. Glomerular filtration rate fell during the first part of the PGI_2 infusion in the salt deplete dogs, probably as a result of the large decrease in systemic blood pressure seen at this time. Glomerular filtration rate increased again towards the end of the PGI_2 infusion, but did not rise above control values.

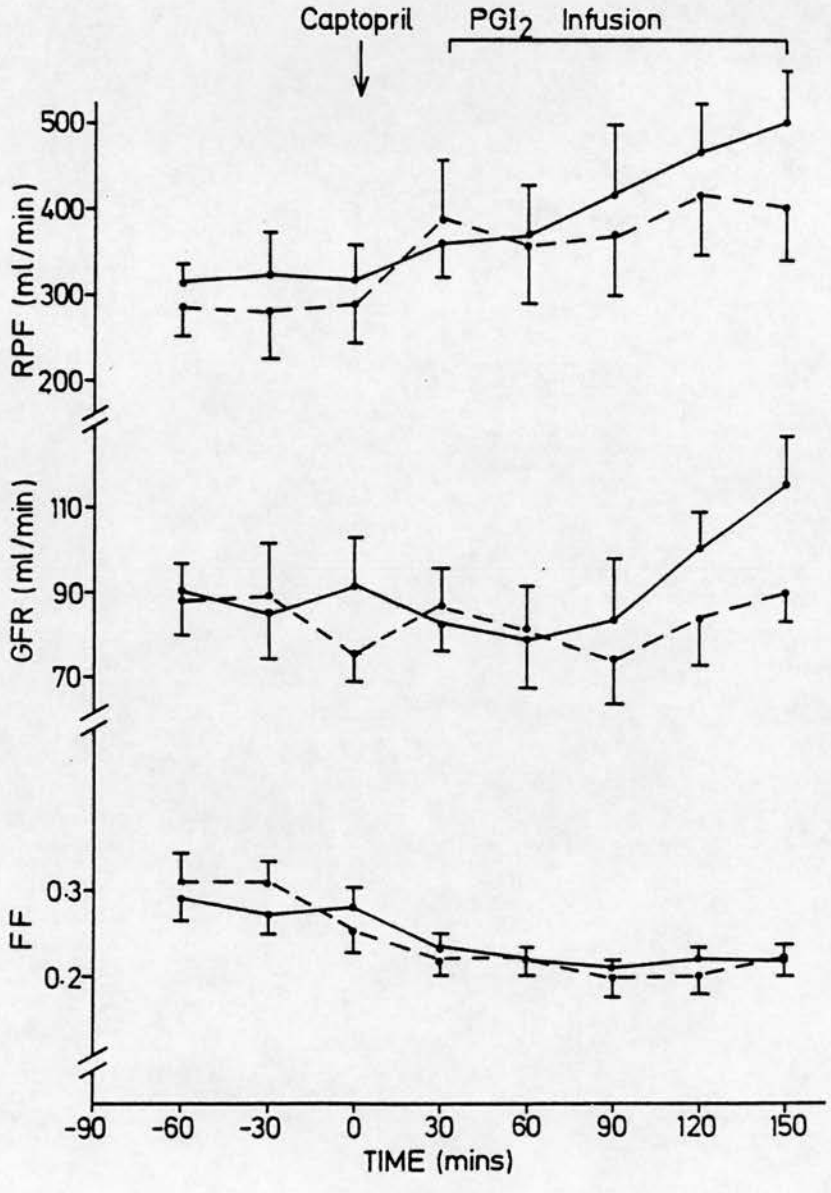


FIGURE 4.8: The effect of captopril administration and intravenous PGI_2 infusion on renal plasma flow, glomerular filtration rate and filtration fraction.

●——● sodium replete dogs;

●- - -● sodium deplete dogs.

4.7.5 Filtration fraction (Fig. 4.8)

Filtration fraction fell after captopril administration in both salt replete and salt deplete dogs due to the increase in renal plasma flow. There was no further decrease in filtration fraction during the PGI_2 infusion due to the increase in glomerular filtration rate during the infusion period.

4.7.6 Sodium excretion (Fig. 4.9)

Sodium excretion was much reduced when the dogs were maintained on the salt restricted diet. In the salt replete dogs sodium excretion increased from a control value of 62 ± 32 μM/min to 89 ± 29 μM/min 30 min after captopril administration. Sodium excretion showed a further progressive increase during the PGI_2 infusion, reaching a maximum value of 196 ± 37 μM/min by the end of the study. In the salt deplete dogs, captopril administration caused sodium excretion to increase from a control value of 2.0 ± 0.5 μM/min to 3.9 ± 0.4 μM/min after administration. In contrast to the effects in the salt replete dogs, sodium excretion showed a small progressive decrease during the PGI_2 infusion, reaching control values by the end of the infusion. This effect may be due to the greatly reduced systemic blood pressure during the PGI_2 infusion in the salt deplete dogs.

4.7.7 Potassium excretion (Fig. 4.9)

Potassium excretion during the control period was not altered by sodium depletion confirming the results in section 4.4.7. In the salt replete dogs, potassium excretion increased from a control value of 37 ± 4 μM/min to 52 ± 8 μM/min 30 min after captopril administration, but showed no further increase during the PGI_2 infusion. Potassium excretion

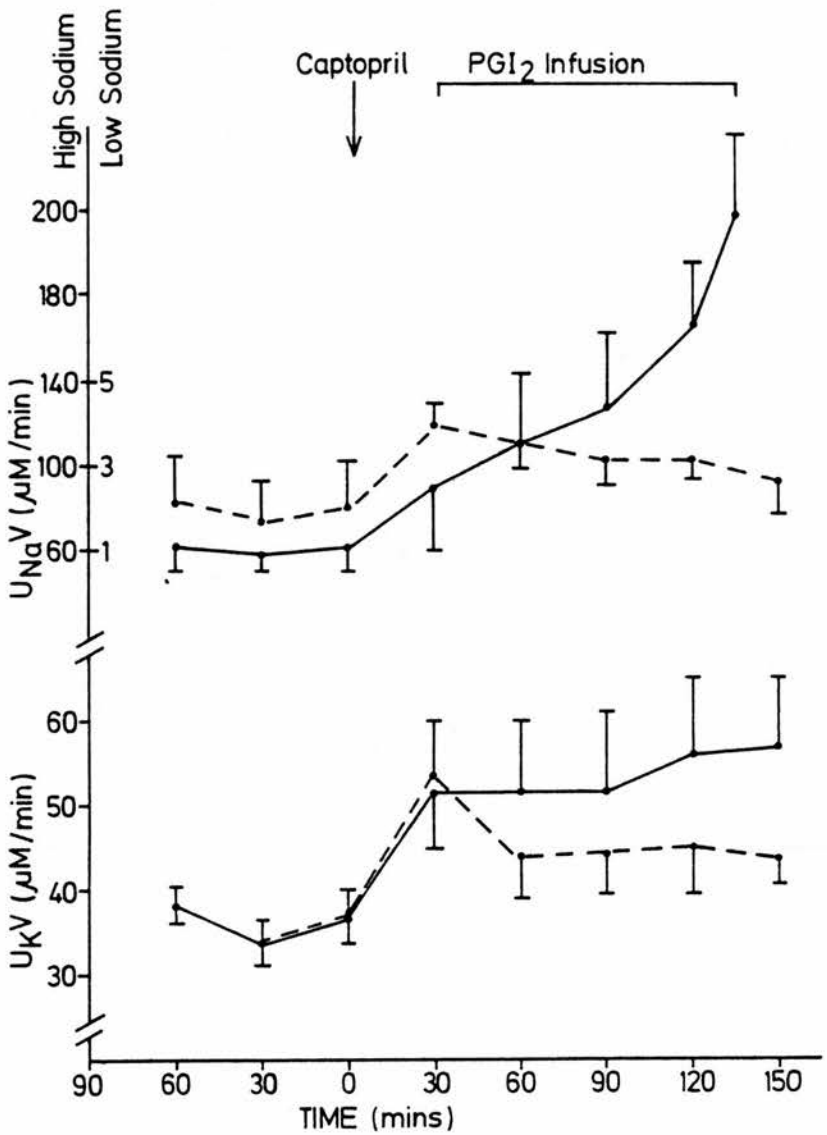


FIGURE 4.9: The effect of captopril administration and intravenous PGI_2 infusion on sodium and potassium excretion.

● ——— ● sodium replete dogs;

● - - - ● sodium deplete dogs.

increased following captopril administration in the salt deplete dogs, from a control value of 37 ± 4 μM/min to 53 ± 7 μM/min 30 min after administration but decreased again to 44 ± 5 μM/min within 30 min of the start of the PGI_2 infusion. This may be as a result of the reduced systemic blood pressure during the PGI_2 infusion in the salt deplete dogs.

4.7.8 Urine flow (Fig. 4.10)

There was a small decrease in urine flow rate after captopril administration and during PGI_2 infusion in the salt replete dogs. No change in urine flow rate was observed in the salt deplete dogs throughout the study.

4.7.9 Solute (osmolality) excretion (Fig 4.10)

Solute excretion during the control periods was slightly lowered by sodium deprivation. Captopril administration had no effect on Solute excretion irrespective of sodium status. Solute excretion increased towards the end of the PGI_2 infusion in the salt replete dogs possibly as a result of the progressive increase in sodium excretion throughout the infusion period. There was no change in osmolality excretion during the PGI_2 infusion in the salt deplete dogs.

4.7.10 Free water clearance (Fig. 4.10)

Free water clearance during the control periods was slightly lowered during sodium deprivation. There was a small decrease in free water clearance after captopril administration and during the PGI_2 infusion in the salt replete dogs but no change in free water clearance was observed in salt deplete dogs.

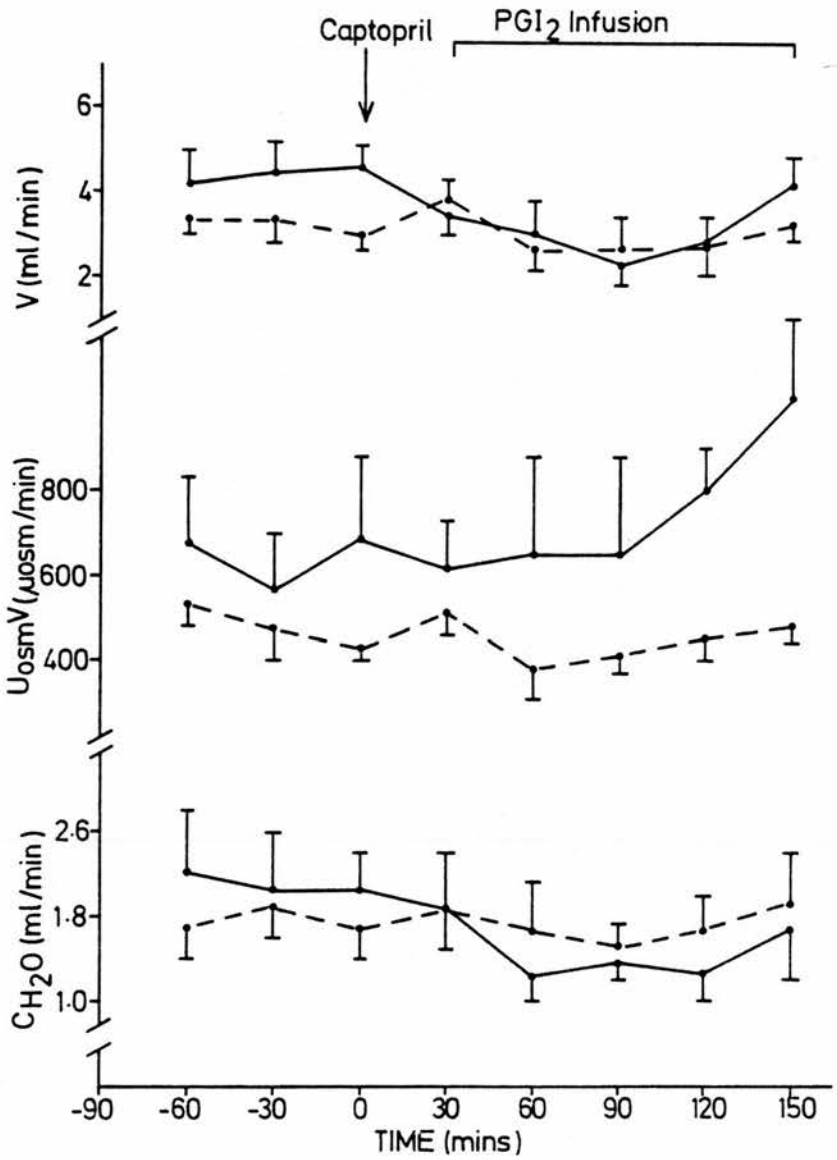


FIGURE 4.10: The effect of captopril administration and intravenous PGI_2 infusion on urine flow, osmolality excretion and free water clearance.

● ——— ● sodium replete dogs;

● - - - ● sodium deplete dogs.

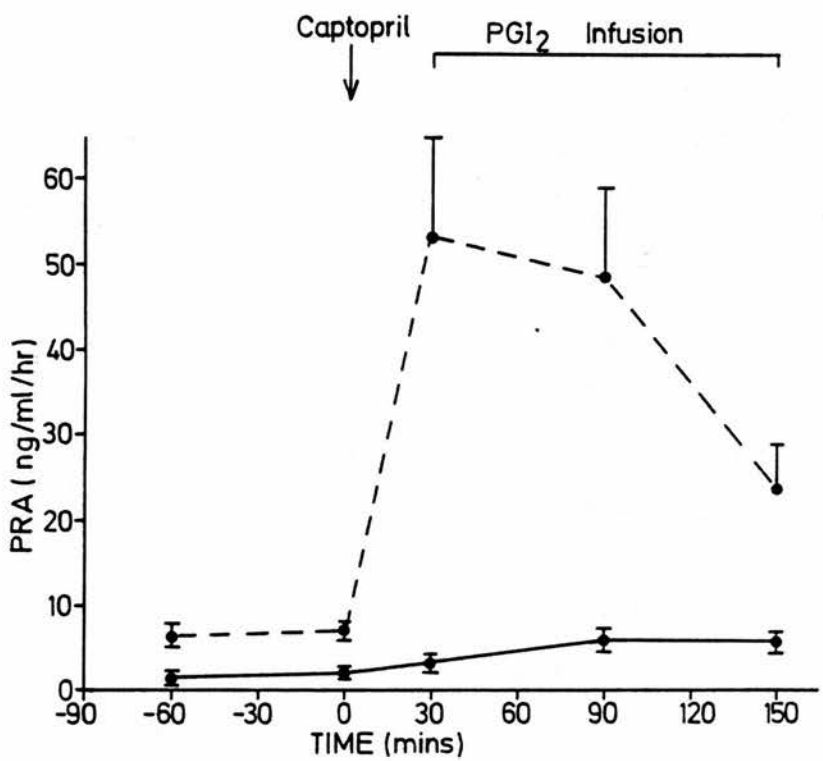


FIGURE 4.11: The effect of captopril administration and intravenous PGI_2 infusion on plasma renin activity.

●——● sodium replete dogs;

●– – –● sodium deplete dogs.

4.7.11 Plasma renin activity (Fig. 4.11)

Control values for plasma renin activity were greatly increased by sodium depletion demonstrating stimulation of the renin-angiotensin system. In the salt replete dogs, plasma renin activity showed a progressive increase after captopril and during PGI_2 infusion, increasing from a control value of 1.9 ± 0.4 ng/ml/hr to 5.0 ± 1.0 ng/ml/hr by the end of the study. In the salt deplete dogs, plasma renin activity increased from a control value of 6.9 ± 0.7 ng/ml/hr to 54 ± 14 ng/ml/hr within 30 min of captopril administration, after which, despite the PGI_2 infusion, it decreased again to 24 ± 5 ng/ml/hr by the end of the study.

4.8 Discussion

Dietary sodium deprivation is used routinely as an effective physiological stimulus for renin release and under such conditions, the renin-angiotensin system becomes important in the control of systemic blood pressure. In the first study in this section, acute dietary sodium restriction in conscious dogs resulted in elevated plasma renin activity and greatly reduced urinary sodium excretion. Despite such stimulation of the renin-angiotensin system, systemic blood pressure was also lowered during sodium restriction. This decrease in blood pressure is most likely due to a fall in extracellular fluid volume which would accompany sodium depletion. The regime of sodium restriction had no effect on heart rate or renal plasma flow.

Inhibition of angiotensin II formation by captopril in the salt deplete dogs resulted in a large fall in systemic blood pressure within 30 min of administration. This demonstrates the importance of angiotensin II in the control of systemic blood pressure under these conditions. Although

the attendant elevation of heart rate suggests that the baroreceptor reflex is intact, blood pressure was not restored and there is evidence to suggest that captopril prevents angiotensin II facilitated adrenergic neurotransmission at the vascular neuroeffector junction thus impairing sympathetic vascular control (Clough, Collis, Conway, Hatton and Veddie, 1982). Systemic blood pressure was also decreased after captopril administration in the salt replete dogs. The fall in blood pressure was maximal within 30 min of captopril administration as in the salt deplete dogs but the decrease in pressure was quantitatively much smaller in the salt replete than in the salt deplete dogs. These results confirm those found in similar studies in dogs and man where captopril administration in the salt replete state resulted in a qualitatively similar but quantitatively smaller reduction in systemic blood pressure to that demonstrated in the salt deplete state (Zimmerman *et al.*, 1980b; MacGregor *et al.*, 1981).

The effect of captopril to decrease systemic blood pressure in sodium replete animals and man is a property not consistently shared with either teprotide or saralasin. This has led to the proposal that stimulation of the synthesis of the vasodilator PGI_2 may be responsible for mediating part of the hypotensive response to captopril.

In the present study, urinary 6-keto-$PGF_{1\alpha}$ has been measured before and after captopril administration to reflect systemic PGI_2 production. Urinary 6-keto-$PGF_{1\alpha}$ excretion increased after captopril administration in both sodium replete and sodium deplete dogs. However, this increase was not apparent until 90 min after captopril administration and was not maintained throughout the study. The difference in time courses between the fall in systemic blood pressure and the increase in urinary 6-keto-$PGF_{1\alpha}$ excretion following captopril administration

make it unlikely that PGI_2 is contributing towards the hypotensive response. An estimation of the rate of PGI_2 synthesis as reflected by the urinary 6-keto-$PGF_{1\alpha}$ excretion can be obtained from the standard curve constructed in section 3.4.12. By this means, estimated rates of PGI_2 synthesis of 3.6 ng/kg/min and 4.3 ng/kg/min were obtained for the sodium replete and sodium deplete dogs respectively, during maximal stimulation by captopril. These levels of PGI_2 in the systemic circulation are well below those required to produce a hypotensive response since an intravenous infusion of PGI_2 of 7.5 ng/kg/min in section 3.4.1 failed to lower systemic blood pressure. It therefore seems extremely unlikely that the small increase in PGI_2 synthesis observed after captopril administration is contributing to the hypotensive action of captopril either in the sodium replete or sodium deplete state. These results, together with the lack of conclusive evidence towards a contribution of other vasodilator mediators in the hypotensive action of captopril suggest that the ability of captopril to lower systemic blood pressure in the salt replete as well as the salt deplete state is directly related to the inhibition of angiotensin II formation. Thus the renin-angiotensin system may exert a tonic influence on the control of systemic blood pressure in the normal, sodium replete state. This is in contradiction with the previously held view that angiotensin II is not a prime factor in blood pressure control under normal circumstances.

Previous studies using saralasin to antagonise the action of angiotensin II found that saralasin lowered systemic blood pressure under conditions of sodium depletion but had a negligible effect on systemic blood pressure under normal circumstances (Hollenberg *et al.*, 1977; Agabiti-Rosei *et al.*, 1979). However, results from such studies are difficult to interpret owing to saralasin's residual agonist activity.

Studies using teprotide have also failed to give consistent results as to the effect of inhibition of angiotensin II formation on systemic blood pressure in the salt replete state. Most investigators have been unable to demonstrate a hypotensive action of teprotide during sodium replete conditions (Kimbrough *et al.*, 1977; Haber, 1976), whilst others have demonstrated an effect similar to that found with captopril (Niarchos *et al.*, 1979). The inconsistency of the results found with teprotide may be partly due to the fact that this drug must be administered intravenously and can therefore only inhibit the short-term effects of angiotensin II. A gradual increase in the importance of the renin-angiotensin system as sodium is lost is more likely than an all-or-none phenomenon and captopril is the first drug to consistently reveal this effect. The results of this study and of others showing a similar hypotensive response to both acute and chronic administration of captopril during sodium repletion (Zimmerman *et al.*, 1980b; Hollenberg *et al.*, 1981; MacGregor *et al.*, 1981) support the view that the renin-angiotensin system exerts a tonic influence on systemic blood pressure under normal conditions.

It has been postulated that angiotensin II produced intrarenally may serve a local regulatory role in the control of renal haemodynamics and sodium excretion (Schmidt, 1962; Thurau, 1963; Guyton, Langston and Navar, 1964). Angiotensin I converting enzyme is present in the kidney (Granger, Dolheim and Thurau, 1972; Caldwell, Seegal and Hsu, 1976) and the presence of angiotensin II in the extravascular compartment of the kidney has been demonstrated (Mendelsohn, 1979). Experiments have shown that intrarenal infusions of subpressor doses of angiotensin II which do not affect circulating angiotensin II levels consistently result in decreased renal blood flow and glomerular filtration rate (Barraclough, Jones and Marsden, 1967; Malvin and Vander, 1967;

Navar and Langford, 1974). Intrarenal infusion of saralasin or teprotide during conditions of sodium depletion lead to an increase in renal blood flow, glomerular filtration rate, and sodium excretion (Trippodo, Hall, Lohmeier and Guyton, 1977; Hall, Coleman, Guyton, Williamson-Balfe and Salgado, 1979). However, a similar effect could not be demonstrated in animals on a normal sodium intake (Lohmeir, Cowley, Trippodo, Hall and Guyton, 1979; Kimbrough *et al.*, 1977). It should be noted that in the studies infusing saralasin or teprotide into sodium deplete animals, the renal effects of these blockers were usually accompanied by a decrease in systemic blood pressure. It is therefore possible that inhibition of circulating as well as renal angiotensin II formation was responsible for the observed effects on renal haemodynamics and sodium excretion.

Although renal and/or circulating angiotensin II is important in the control of renal haemodynamics and sodium excretion during sodium depletion, controversy still exists as to the precise role of the renin-angiotensin system in controlling renal function during conditions of normal sodium intake. In the present study, captopril administration results in an increase in renal plasma flow in the salt deplete and salt replete states without causing any significant change in glomerular filtration rate. In both the salt replete and deplete dogs, the response of renal plasma flow to captopril was transient, reaching a maximum 90 min after drug administration. Captopril caused a somewhat larger increase in renal plasma flow in the salt deplete dogs than in the salt replete dogs. This difference in vasodilator response was, however, of a much lesser magnitude than the difference in hypotensive response to captopril seen between the salt replete and salt deplete dogs. These results are in agreement with those found by other investigators studying the effects

of captopril in normotensive and hypertensive dogs (Zimmerman *et al.*, 1980a) and suggest that under normal conditions, the renin-angiotensin system plays a role in the control of renal haemodynamics, the degree of control increasing during sodium depletion.

Filtration fraction remained unchanged after captopril administration in the sodium replete dogs but decreased in the sodium deplete dogs due to the larger increase in renal plasma flow. This suggests that, at least in the sodium deplete state, endogenous angiotensin II controls renal haemodynamics by preferential constriction of the efferent arterioles. In the present study, it is not possible to determine whether the renal vasodilatation following captopril administration was due to inhibition of intrarenal or circulating angiotensin II formation.

The renal vasodilatation following captopril administration can be explained solely by the inhibition of a tonic influence of angiotensin II on renal haemodynamics. However, there is a close correlation between the increase in renal plasma flow and urinary 6-keto-$PGF_{1\alpha}$ excretion in both sodium replete and sodium deplete dogs. Urinary 6-keto-$PGF_{1\alpha}$ excretion was increased to a slightly greater extent in the salt deplete dogs following captopril administration than it was in the salt replete dogs. This corresponds to the somewhat larger increase in renal plasma flow and suggests that PGI_2 may be mediating part of the renal vasodilator response to captopril. Since even the maximum urinary 6-keto-$PGF_{1\alpha}$ excretion rate reflects low levels of systemic PGI_2, it is unlikely that the increase in PGI_2 production is the sole mediator of the renal vasodilator response to captopril. However, it is possible that PGI_2 is contributing to the renal vasodilator action of captopril, particularly if the urinary 6-keto-$PGF_{1\alpha}$ is reflecting predominantly increased renal synthesis of PGI_2. The demonstration that indomethacin caused a small

but non-significant attenuation of the renal vasodilator response to captopril (Wong *et al.*, 1981) supports the proposal that PGI_2 is playing a minor role in the renal vasodilation. A recent study investigating the contribution of PGE_2 to the renal vasodilator response to captopril in anaesthetised dogs demonstrated an increase in renal venous PGE_2 concentration following captopril administration (Oliver, Sciacca and Cannon, 1983). The same study failed to demonstrate any effect of indomethacin on the renal vasodilator response to captopril and the investigators concluded that although captopril resulted in an increase in PGE_2 synthesis, this prostaglandin was not contributing to the observed renal vasodilatation. The results of the present study demonstrate that PGI_2 synthesis is increased following captopril administration and that this increase can be closely correlated with renal plasma flow. Whilst this suggests a contribution of PGI_2 to the renal vasodilator action of captopril, such a conclusion cannot be made from the present results.

Captopril caused an increase in sodium excretion in both sodium deplete and sodium replete dogs. The ability of captopril to cause an increase in sodium excretion in the salt replete dogs confirms the finding that chronic captopril administration increases sodium excretion in normal man (MacGregor *et al.*, 1981). This is in disagreement with earlier studies with saralasin and teprotide which failed to demonstrate an effect on sodium excretion in sodium replete conscious dogs (Hall, Guyton, Trippodo, Lohmeier, McCaa and Cowley, 1977; Kimbrough *et al.*, 1977). Free water clearance fluctuated throughout the study but there was no significant change after captopril administration which would suggest that sodium reabsorption has been inhibited in the proximal tubule. The decrease in free water clearance at the end of the study in the salt replete dogs may reflect some inhibition of distal tubular

sodium reabsorption. Potassium excretion also fluctuated after captopril administration but tended to increase in both sodium replete and deplete dogs. This increase in potassium excretion also suggests that sodium reabsorption has been inhibited in the proximal tubule resulting in more sodium being delivered to the distal tubule to enter into an exchange mechanism with potassium. The increase in sodium excretion may be due, at least in part, to the increase in renal plasma flow. An increase in renal plasma flow without a concomitant increase in glomerular filtration rate is capable of increasing sodium excretion, primarily by increasing peritubular capillary hydrostatic pressure. There may also have been a redistribution of intrarenal blood flow which would affect sodium reabsorption. It seems unlikely however that the increase in sodium excretion is solely a consequence of the increase in renal blood flow, particularly in the sodium replete dogs where the natriuresis following captopril administration is large. PGI_2 is capable of causing natriuresis (Gerber *et al.*, 1978a). However, an increase in sodium excretion was observed within 30 min of captopril administration in both sodium replete and deplete states while urinary 6-keto-$PGF_{1\alpha}$ was not increased until 90 min. It is also unlikely that the degree of stimulation of PGI_2 synthesis is large enough to exert such a significant effect on sodium excretion. The renin-angiotensin system has a known ability to conserve sodium by stimulating aldosterone release. Inhibition of angiotensin II formation by captopril would result in lowered aldosterone levels which could result in increased sodium excretion at least in the salt deplete animal. Infusion of aldosterone during chronic captopril administration in conscious salt deplete dogs, however, fails to prevent the increase in sodium excretion (McCaa *et al.*, 1978; Hall *et al.*, 1979b). It is unlikely that decreased aldosterone release is responsible for the natriuresis observed after

captopril administration in the salt replete dogs since under normal conditions, plasma aldosterone levels are low and do not exert a large influence on sodium reabsorption. Results from micropuncture studies suggest that angiotensin II exerts a direct effect on the proximal tubule to stimulate sodium reabsorption (Harris and Young, 1977) although this effect could only be demonstrated with low doses of angiotensin II, higher doses resulting in inhibition of sodium reabsorption. This dose-dependant dual action of angiotensin II on sodium reabsorption has also been demonstrated in isolated proximal tubules (Freedlender and Goodfriend, 1977) and high and low affinity binding sites for angiotensin II in the proximal tubule have been identified (Freelander, Kirschmaum, Ris, Oken, and Candelora, 1980). Thus the large natriuresis observed after captopril administration in the salt replete dogs may be partly due to the removal of a direct effect of angiotensin II on the tubule to increase sodium reabsorption. Were angiotensin II to exhibit a bell-shaped dose-response curve for sodium excretion then this may explain the less dramatic effect of captopril on sodium excretion in the salt deplete dogs. During sodium depletion, angiotensin II levels will be high and consequently may be acting on the downward slope of the dose-response curve. In this situation, inhibition of angiotensin II by captopril would not be expected to have such a large effect on sodium excretion as during sodium repletion when low levels of angiotensin II were acting on the upward slope of the dose response curve. This would imply that during sodium depletion, other factors besides angiotensin II are responsible for conservation of sodium.

The response of plasma renin activity to captopril administration was much more pronounced in the salt deplete than in the salt replete dogs. Plasma renin activity showed a small but progressive increase

following captopril administration in the salt replete dogs but showed a large increase, maximal within 30 min of drug administration in the salt deplete dogs. This initial large increase in plasma renin activity in the salt deplete dogs can be partly attributed to stimulation of the renal baroreceptor mechanism due to the reduction in systemic blood pressure following captopril administration. Angiotensin II inhibits renin release by a short-loop negative feedback mechanism (Vander and Geelhoed, 1965; Bunag *et al.*, 1967) and therefore the rate of renin secretion is the result of the strength of the stimulus causing release and the degree of inhibition of release exerted by angiotensin II. Thus with the removal of the negative feedback influence of angiotensin II by captopril, renin release will increase due to the stimulus of sodium depletion. In the sodium replete dogs, where there is no obvious stimulus for renin release, removal of the negative feedback influence of angiotensin II results only in a very modest increase in renin release. Although PGI_2 can increase renin release from the kidney (Whorton *et al.*, 1981), it seems unlikely that it is responsible for the increased plasma renin activity observed following captopril administration. The increase in plasma renin activity in the salt deplete dogs occurs well before the observed increase in urinary 6-keto-$PGF_{1\alpha}$. Although PGI_2 may be contributing to the increased renin release in the salt replete dogs, it is not the sole factor since plasma renin activity is still increased at the end of the study when urinary 6-keto-$PGF_{1\alpha}$ excretion has returned to control levels. In addition, the degree of stimulation of PGI_2 synthesis is unlikely to be sufficient to elicit a significant increase in plasma renin activity since an intravenous infusion of a substantially higher concentration of PGI_2 in section 3 failed to have any effect on plasma renin activity. Previous studies with indomethacin in normotensive and hypertensive

animals and man have yielded conflicting results as to whether prostaglandins are involved in the increased renin release evoked by captopril (Miyamori *et al.*, 1980; Salvetti *et al.*, 1980; Abe *et al.*, 1979; Silberbauer *et al.*, 1982). However, recent studies support the results of the present investigation and suggest that, at least in the dog, PGI_2 does not mediate the increase in plasma renin activity following captopril administration either during sodium repletion or sodium depletion (De Forrest, Waldron and Antonaccio 1982; Oliver *et al.*, 1983).

It is not possible in this study to determine whether the increased PGI_2 synthesis, as reflected by the urinary 6-keto-$PGF_{1\alpha}$ excretion, is due to a direct action of captopril or whether it is a consequence of increased bradykinin levels. The fact that PGI_2 synthesis was increased in the sodium deplete as well as the sodium replete states suggests that the higher angiotensin II levels during sodium depletion were not causing stimulation of PGI_2 synthesis since had this been the case, inhibition of angiotensin II formation by captopril would have resulted in decreased PGI_2 synthesis. The evidence for stimulation of PGI_2 synthesis by angiotensin II is conflicting and the majority of studies have used *in vitro* preparations and high concentrations of angiotensin II. Some studies have demonstrated increased release of PGI_2 from isolated lung (Grodzinska and Gryglewski, 1980), mesentery (Dusting *et al.*, 1981), heart (Needleman *et al.*, 1978), and kidney (Shebuski and Aiken, 1980). Other studies have failed to demonstrate a stimulation of PGI_2 synthesis by angiotensin II in porcine aortic endothelial cells (Whorton, Young, Data, Barchowsky and Kent, 1982) or rabbit kidney (Needleman, Bronson, Wyche and Sivakoff, 1978).

Studies investigating renal PGE_2 synthesis during stimulation of renin release and increased angiotensin II formation due to sodium depletion have produced conflicting results. In the rat, an inverse relationship was found between renal tissue PGE_2 production and sodium intake (Tobian and O'Donnel, 1976) and in the dog, renal venous PGE_2 levels increased following sodium restriction (Oliver *et al.*, 1980). An increase in urinary PGE_2 excretion has been demonstrated following sodium restriction in the rabbit (Stahl, Ahmad, Attallah, Bloch and Lee, 1979) and in man (Rathaus *et al.*, 1981) whilst other investigators have failed to observe any change in urinary PGE_2 excretion due to sodium depletion in the rabbit (Lifschitz *et al.*, 1978) or dog (Blasingham *et al.*, 1980) or in plasma PGE_2 levels in man (Zusman, Spector, Cadwell, Speroff, Schneider and Mulrow, 1973). Studies with indomethacin or meclofenamate have revealed that prostaglandin synthesis inhibition results in an enhanced pressor and renal vasoconstrictor response to angiotensin II (Negus *et al.*, 1976; Aiken and Vane, 1973). It has also been demonstrated that prostaglandin synthesis inhibition results in a decrease in renal blood flow in the sodium depleted conscious dog but has no effect when animals are sodium replete (Zimmerman, Momsen and Kraft, 1980b; De Forrest *et al.*, 1980). This suggests that renal prostaglandins help to maintain renal blood flow when angiotensin II levels are high. The ability of indomethacin or meclofenamate to decrease renal blood flow during sodium depletion in the absence of convincing evidence that PGE_2 production is increased has led some investigators to postulate that PGI_2 is the important prostaglandin in maintaining renal blood flow during stimulation of the renin-angiotensin system (Blasingham *et al.*, 1980). The failure to observe an elevation of the urinary 6-keto-$PGF_{1\alpha}$ excretion in the salt deplete state during the control periods in the present study

suggests that this is not the case and it would seem that a reappraisal of the role of prostaglandins in the control of renal haemodynamics is necessary.

The results of this first study demonstrate that although PGI_2 may contribute to the renal vasodilator response, the hypotensive, renal vasodilator and natriuretic actions of captopril are due primarily to inhibition of angiotensin II formation during both sodium deplete and replete states. The ability of captopril to lower systemic blood pressure and cause marked increases in renal blood flow and sodium excretion in the salt replete dogs suggest that under normal conditions, the renin-angiotensin system is an important factor in the control of blood pressure and renal function.

The large vasodilator and natriuretic responses to captopril have made it difficult to draw firm conclusions in the second study of this section since many of the expected actions of PGI_2 have been masked by the consequent effects of inhibition of angiotensin II formation. Captopril elicited a hypotensive response within 30 min in both salt replete and salt deplete dogs. The responses were quantitatively similar to those found in the previous study. PGI_2 infusion resulted in a further small decrease in systemic blood pressure in both the sodium replete and deplete dogs. The PGI_2-induced hypotension following captopril administration was not so pronounced as that produced by the same concentration of PGI_2 infused into dogs without prior treatment with captopril (see section 3.4.1, Figure 3.1). The fall in systemic blood pressure during PGI_2 infusion was transient, a maximum hypotension occurring 60-90 min after the start of the infusion, an effect also seen in section 3.4.1. By the end of the study, despite continued PGI_2 infusion, blood pressure had returned to the levels seen following captopril administration.

Chronic infusion of PGE_2 into the renal artery of dogs results in a decrease in systemic blood pressure followed by a sustained hypertension (Hockel and Cowley, 1980). This was thought to be a result of a stimulation of renin release with a consequent increase in angiotensin II formation. It was thought that a similar mechanism may be responsible for the failure of PGI_2 infusion to cause a sustained fall in systemic blood pressure. The lack of a significant increase in plasma renin activity during PGI_2 infusion together with an unsustained hypotension even when angiotensin II formation was inhibited by captopril suggests that stimulation of the renin-angiotensin system was not responsible for the transient effect of PGI_2 on systemic blood pressure.

The infusion of PGI_2 following captopril administration produced a larger fall in systemic blood pressure in the sodium replete than in the sodium deplete state. This may be due to the fact that in the sodium deplete dogs, captopril caused greater vasodilatation of the vasculature. PGI_2 infusion also increased renal plasma flow to a greater extent in sodium replete dogs than in sodium deplete dogs although prior captopril administration had resulted in the same degree of renal vasodilatation in both sodium replete and deplete states. In the sodium replete dogs both renal plasma flow and glomerular filtration rate showed a progressive increase during the latter half of the PGI_2 infusion. Since renal plasma flow only showed a transient increase and glomerular filtration rate did not change at all after captopril administration in the previous study, the increases seen in the latter half of the present study can be attributed to the effects of PGI_2. The increase in renal plasma flow and glomerular filtration rate during the PGI_2 infusion are larger than would be expected from the results in section 3.4.3, Figure 3.2, where the same concentration of PGI_2 elicited a smaller effect on renal plasma

flow and no change in glomerular filtration rate. It is possible therefore that inhibition of angiotensin II formation by captopril has allowed PGI_2 to have a greater vasodilator action on the renal vasculature. Although filtration fraction decreased after captopril administration, the increase in glomerular filtration rate prevented a further decrease during the PGI_2 infusion. This may indicate that the PGI_2 is having a preferential vasodilator action on the afferent arteriole. Alternatively, inhibition of angiotensin II formation by captopril may have interfered with autoregulatory feedback control of glomerular haemodynamics such that any increase in renal plasma flow results in an increase in glomerular filtration rate.

The inability of PGI_2 to produce such changes in renal haemodynamics in the salt deplete dogs cannot be explained simply by the effect of prior captopril administration since captopril caused the same degree of renal vasodilatation in both sodium replete and deplete states. The effect of exogenous PGI_2 to cause vasodilatation may be related to the number of free receptors. It has been demonstrated that the vasoconstrictor response to exogenous angiotensin II is decreased during sodium depletion due to increased prior receptor occupancy by the elevated plasma angiotensin II levels (Brunner *et al.*, 1962; Thurston and Laragh, 1975). The urinary 6-keto-$PGF_{1\alpha}$ excretion data in the previous study demonstrated that PGI_2 production in the salt deplete dogs was increased to a slightly higher level by captopril than it was in the salt replete dogs. It is possible therefore, that increased prior receptor occupancy is responsible for the lack of further renal vasodilatation during the PGI_2 infusion in the sodium deplete dogs. However, it is also possible that sodium depletion results in the vasculature being less sensitive to the effects of PGI_2 by some alternative mechanism.

Sodium excretion showed a large increase during the PGI_2 infusion in the sodium replete dogs, although this effect could be due solely to the effects of captopril administration since the previous study demonstrated that captopril administration alone results in a progressive increase in sodium excretion (Figure 4.3). The maximum increase in sodium excretion is higher during the PGI_2 infusion following captopril administration than that observed following captopril administration alone. This suggests that the PGI_2 may be having an additive effect to increase sodium excretion. Although the increase in sodium excretion could be explained by the increased renal plasma flow resulting in decreased proximal tubular reabsorption, the lack of increase in potassium excretion and the decrease in free water clearance suggest that distal tubular sodium reabsorption is also being inhibited. No such effect on distal tubular sodium reabsorption was demonstrated either by captopril alone (section 4.5.7) or by the infusion of the same concentration of PGI_2 without prior captopril administration (section 3.5.7). Thus inhibition of angiotensin II formation by captopril may have allowed the PGI_2 to exert an inhibitory effect on distal tubular sodium reabsorption. It is unlikely, however, that this is a direct effect on the distal tubule since no further increase in sodium excretion was demonstrated during PGI_2 infusion in sodium deplete dogs when renal plasma flow also failed to increase.

PGI_2 infusion following captopril administration had no effect on plasma renin activity in the salt replete dogs. This demonstrates that the removal of the negative feedback influence of angiotensin II in no way augments the effect of PGI_2 on renin release. The increase in plasma renin activity following captopril administration in the salt deplete dogs was so large that any effect of PGI_2 infusion would be completely masked.

Both studies in this section have demonstrated that inhibition of angiotensin II formation by captopril causes large changes in systemic blood pressure, renal blood flow and sodium excretion in both salt replete and salt deplete dogs indicating the importance of the renin-angiotensin system in the control of these parameters. In the first study, the estimated maximum rates of synthesis of PGI_2 following captopril administration were 3.6 ng/kg/min and 4.3 ng/kg/min for the salt replete and salt deplete dogs respectively. By comparison to the effects of intravenous infusion of PGI_2 in Section III these levels were not thought to be high enough to exert an effect on systemic haemodynamics. However, had captopril administration resulted in a large increase in vascular sensitivity to PGI_2, these levels may have been sufficient to result in vasodilatation. Inhibition of angiotensin II formation by captopril had no effect on the hypotensive response to PGI_2 although it did result in a slightly greater renal vasodilator response to PGI_2 in the sodium replete dogs. Thus whilst the low levels of PGI_2 demonstrated in the first study were unlikely to have an effect on systemic blood pressure even after inhibition of angiotensin II formation by captopril, it is possible that they were sufficient to participate in the renal vasodilatation. It should be noted, however, that the large effects of prior captopril administration make it difficult to draw any firm conclusions.

The lack of effect of PGI_2 on renal plasma flow following captopril administration in the salt deplete dogs, in addition to the finding that urinary 6-keto-$PGF_{1\alpha}$ was not elevated during the control period of sodium deprivation, suggests that PGI_2 is not important in maintaining renal blood flow when the renin-angiotensin system is stimulated by sodium depletion. The failure of PGI_2 to increase renin release after removal of the negative feedback influence of angiotensin II also questions

the significance of PGI_2 in the control of renin release. It would seem, therefore, that in comparison to angiotensin II, PGI_2 plays a very minor role in the control of systemic blood pressure and renal function in either the sodium replete or sodium deplete dog.

SECTION V

General Discussion

The aim of this thesis was to investigate the complex interrelationship between PGI_2 and the renin-angiotensin system. This was achieved firstly by observing the actions of exogenous PGI_2 on renal parameters and secondly by interrupting the renin-angiotensin system and demonstrating whether or not this affected endogenous synthesis of PGI_2 or the expected actions of exogenous PGI_2.

The use of conscious animals throughout this work proved to be of great benefit. The disadvantages were that the surgical preparation and training of each dog took 2 months and the maintenance of each animal in a healthy state required constant attention. However, these disadvantages were offset, firstly by the obvious advantage of being able to study the prostaglandin and renin-angiotensin systems without the use of anaesthesia. Secondly, once initiated into an experimental regime, each dog could be used repeatedly for a number of different studies, thereby acting as its own control. In this thesis, therefore, the same animals were used in all studies and it was this advantage which made it possible to make direct comparisons between the studies.

Endogenous PGI_2 production was assessed by estimation of urinary 6-keto-$PGF_{1\alpha}$. The linear relationship observed between intravenous PGI_2 and urinary 6-keto-$PGF_{1\alpha}$ demonstrated that measurement of urinary 6-keto-$PGF_{1\alpha}$ represented a useful and reliable indicator of PGI_2 production. The GC-MS assay technique proved to be a reliable and highly specific method and was therefore ideally suited for the precise analytical work required in this thesis, particularly in the demonstration of the relationship between PGI_2 and its metabolite. However, GC-MS is not as sensitive as some recently developed radioimmunoassays and the chromatographic steps necessary to render the urine samples suitable for analysis by GC-MS were found to be time-consuming and laborious.

The author does therefore not consider GC-MS to be a practical alternative to RIA for the estimation of prostaglandins when large sample size is involved.

This thesis has confirmed that the renin-angiotensin system is a very powerful humoral mechanism controlling systemic and renal haemodynamics to varying degrees depending on the state of sodium balance. Although it would be speculative to say that under physiological conditions, PGI_2 plays no role in the control of systemic or renal haemodynamics, it is apparent that its contribution to the control of these parameters is minor compared to that of the renin-angiotensin system. Likewise, it is highly unlikely that PGI_2 is an important modulator of the renin-angiotensin system although it may act as a 'fine-control', preventing dramatic fluctuations in renin release and affording some local protection of blood flow within the kidney at times when angiotensin II formation is increased.

Since the discovery of prostaglandins by Von Euler in 1963, the literature has witnessed a vast quantity of evidence implicating prostaglandins in almost every physiological system in the body. Perhaps the enthusiasm which the discovery of a new group of compounds inspired, led to the role of prostaglandins as modulators of many body functions being given an exaggerated importance. Although there can be no doubt that prostaglandins are involved in many physiological processes, the fact that millions of people ingest pharmacological doses of aspirin every day with few apparent side-effects, suggests that the involvement of prostaglandins in these processes is not essential.

This thesis has demonstrated that PGI_2 plays a much less important role in the control of renal function and the renin-angiotensin system than the literature previously suggested. This is an observation which could perhaps be applied to prostaglandin research in general and

suggests that it is time to reassess the relevance of much of the work in this field.

The development of angiotensin converting enzyme inhibitors such as captopril has highlighted our incomplete knowledge of the complexities of the renin-angiotensin system and further study of this important humoral mechanism is essential.

REFERENCES

ABE, K., ITOH, T., SATOH, M., HARUYAMA, T., IMAI, Y., GOTO, T., SATOH, K., OTSUKA, Y. and YOSHINAGA, K. (1979). Life Sci. 26: 561-565.

ABE, K., ITOH, T., IMAI, Y., SATOH, M., HARUYAMA, T., SAKURAI, Y., GOTO, T., OTSUKA, Y. and YOSHINAGA, K. (1980). Jpn. Circ. J. 44: 422-425.

AGABITI-ROSEI, E., BROWN, E., BROWN, J.J., FRASER, W.C.B., TRUST, R., LEVER, A.F., MORTON, J.J. and ROBERTSON, J.I.S. (1979). Clin. Endocrinol. 10: 227-234.

AIKEN, J.W. and VANE, J.R. (1973). J. Pharmacol. Exp. Ther. 184: 678-688.

AITKINSON, A.B., BROWN, J.J., FRASER, R., LECKIE, B., LEVER, A.F., MORTON, J.J. and ROBERTSON, J.I.S. (1979). Clin. Sci. 57: 139S-143S.

ANDERSON, R.J., BERL, T., McDONALD, K.M. and SCHRIER, R.W. (1975). J. Clin. Invest. 56: 420-426.

ANDERSON, G.H., STREETON, D.H.P. and DALAKOS, T.G. (1977). Circ. Res. 40: 243-250.

ANEES, M., SWEET, P., TAN, S.Y. and MULROW, P.J. (1979). Prostglandins. 17: 515-524.

ANGGARD, E., LARSSON, C. and SAMUELLSON, B. (1971). Acta. Physiol. Scand. 81: 396-404.

ANGGARD, E., BOHMAN, S.O., GRIFFIN, J.E., LARSSON, C. and MAUNSBACH, A.B. (1972). Acta. Physiol. Scand. 84: 231-239.

ANTONNACIO, M.J., HARRIS, D., GOLDENBERG, H., HIGH, J.P. and RUBIN, B. (1979). Proc. Soc. Exp. Biol. Med. 162; 429-435.

ARMSTRONG, J.M., DUSTING, G.J., MONCADA, S. and VANE, J.R. (1978). Circ. Res. 42: 112-119.

ATTALLAH, A.A., STAHL, R.A.K., BLOCH, D.L., AMBRUS, J.L. and LEE, J.B. (1982). Amer. J. Cardiol. 49: 1521-1523.

BAER, P.G. and McGIFF, J.C. (1979). Eur. J. Pharmacol. 54: 359-363.

BAKHLE, V.S. (1968). Nature. 220: 19-21.

BARRACLOUGH, M.A. and MILLS, I.H. (1965). Clin. Sci. 28: 69-74.

BARRACLOUGH, M.A., JONES, N.F. and MARSDEN, C.D. (1967). Amer. J. Physiol. 212: 1153-1157.

BECK, N.P., KANETO, T., ZEOR, V., FIELD, J.B. and DAVIS, B.B. (1971). J. Clin. Invest. 50: 2461-2465.

BEIERWALTES, W.H., SCHRYVER, S., OLSON, P.S. and ROMERO, J.C. (1980). Amer. J. Physiol. 239: F602-F608.

BEILBY, D.S., COGHLAN, J.P., DENTON, D.A., GRAHAM, W.F., HUMPHREY, T.J., SCOGGINS, B.A. and WHITWORTH, J.A. (1981). Clin. Exp. Pharmacol. Physiol. 8: 33-37.

BERGSTRÖM, S., RYHAGE, R., SAMUELSSON, B. and SJOVALL, J. (1963). J. Biol. Chem. 238: 3555-3564.

BERL, T., HENRICH, W.L., ERICKSON, A.L. and SCHRIER, R.W. (1979). Amer. J. Phsyiol. 236: F472-F477.

BLACKSHEAR, J.L., SPIELMAN, W.S., KNOX, F.G. and ROMERO, J.C. (1979). Amer. J. Physiol. 237: F20-F24.

BLACKWELL, G.J., FLOWER, R.J. and VANE, J.R. (1975). Biochem. Biophys. Acta. 398: 178-190.

BLAIR, I.A., BARROW, S.E., WADDELL, K.A., LEWIS, P.J. and DOLLERY, C.T. (1982). Prostaglandins. 23: 579-589.

BLASINGHAM, M.C. and NASJLETTI, A. (1979). Amer. J. Physiol. 237: F182-F187.

BLASINGHAM, M.C. and NASJLETTI, A. (1980)a Amer. J. Physiol. 239: F360-F365.

BLASINGHAM, M.C., SHADE, R.E., SHARE, L. and NASJLETTI, A. (1980)b J. Pharmacol. Exp. Ther. 214: 1-4.

BLUMBERG, A.L., DENNY, S.E., MARSHALL, G.R. and NEEDLEMAN, P. (1977). Amer. J. Physiol. 232: H305-H310.

BOBERG, U., HAHNE, B. and PERSSON, A.E.G. (1981). VIII Int. Congr. Nephrol. Abst. 11.

BOLGER, P.M., EISNER, G.M., RAMWELL, P.W. and SLOTKOFF, L.M. (1976). Nature. 259: 244-245.

BOLGER, P.M., EISNER, G.M., SHEA, P.T., RAMWELL, P.W. and SLOTKOFF, L.M. (1977). Nature. 267: 628-630.

BOLGER, P.M., EISNER, G.M., RAMWELL, P.W. and SLOTKOFF, L.M. (1978). Nature. 271: 467-469.

BROWN, J.J., DAVIES, D.L., LEVER, A.F. and ROBERTSON, J.I.S. (1963). Lancet i. 278-279.

BRUBACHER, E.S. and VANDER, A.J. (1968). Amer. J. Physiol. 214: 15-21.

BRUNNER, H.R., CHANG, P., WALLACH, R., SEALEY, J.E. and LARACH, J.H. (1962). J. Clin. Invest. 51: 58-67.

BUNAG, R.D., PAGE, I.H. and McCUBBIN, J.W. (1967). Cardiovasc. Res. 1: 67-73.

CALDWELL, P.R.B., SEEGAL, B.C. and HSU, H.C. (1976). Sci. 191: 1050-1051.

CAMPBELL, W.B., GRAHAM, R.M. and JACKSON, E.K. (1979). J. Clin. Invest. 64: 448-456.

CARRETERO, O.A. and SCICLI, A.G. (1980). Amer. J. Physiol. 238: F247-F251.

CHANG, L.C., SPLAWINSKI, J.A., OATES, J.A. and NIES, A.S. (1975). Circ. Res. 36: 204-207.

CHASSIS, H., RANGES, H.A., GOLDRING, W. and SMITH, H.W. (1938). J. Clin. Invest. 17: 683-697.

CHO, M.J. and ALLEN, M.A. (1978). Prostaglandins. 15: 943-954.

CODY, R.J., TARAZI, R.C., BRAVO, E.L. and FOUAD, F.M. (1978). Clin. Sci. Mol. Med. 55: 453-459.

COOTE, J.H., JOHNS, E.J., MacLEOD, V.H. and SINGER, B. (1972). J. Physiol. 226: 15-36.

CRANTZ, F.R., SWARTZ, S.C., HOLLENBERG, N.K., MOORE, T.J., DLUHY, R.G. and WILLIAMS, G.H. (1981). Hypertension. 2: 604-609.

CROWSHAW, K. and SZLYK, J.Z. (1970). Biochem. J. 116: 421-424.

CUSHMAN, D.W., CHEUNG, H.S., SABO, E.F. and ONDETTI, M.A. (1977). Biochem. 16: 5484-5491.

CUTHBERT, M.F. (1973). In: "The Prostaglandins". ed. Cuthbert, M.F. 1-26.

DANIELS, E.G., HINMAN, J.W., LEACH, B.E. and MUIRHEAD, E.E. (1967). Nature. 215: 1298.

DANON, A., CHANG, L.C.T., SWEETMAN, B.J., NIES, A.S. and OATES, J.A. (1975). Biochem. Biophys. Acta. 388: 71-83.

DATA, J.L., GERBER, J.G., CRUMP, W.J., FROLICH, J.C., HOLLIFIELD, J.W. and NIES, A.S. (1978). Circ. Res. 42: 454-458.

DAVIS, J.O. and FREEMAN, R.H. (1976). Physiol. Rev. 56: 1-56.

De FORREST, J.M., DAVIS, J.O., FREEMAN, R.H., SEYMOUR, A.A., RAVE, B.P., WILLIAMS, G.M. and DAVIS, T.P. (1980). Circ. Res. 47: 99-107.

De FORREST, J.M., WALDRON, T.L. and ANTONACCIO, M.J. (1982). Amer. J. Physiol. 243: F543-F547.

* CLOUGH, D.P., COLLIS, M.G., CONWAY, J., HATTON, R. and KEDDIE, J.R. (1982). Amer. J. Cardiol. 49: 1410 – 4.

* DAWBORN, J.K. (1965). Clin. Chim. Acta. 12: 63 – 66.

DEW, M.E. and MICHELAKIS, A.M. (1974). Pharmacologist. 16: 198.

Di NICOLANTONIO, R., DUSTING, G.J., HUTCHISON, J.S. and MENDELSOHN, F.A. (1981). Clin. Exp. Pharmacol. Physiol. 8: 345-349.

DUNHAM, E.W. and ZIMMERMAN, B.G. (1970). Amer. J. Physiol. 219: 1279-1285.

DUNN, M.J., LIARD, J.F. and DRAY, F. (1978). Kidney Int. 13: 136-143.

DUSTING, G.J., MONCADA, S. and VANE, J.R. (1978). Brit. J. Pharmacol. 64: 315-320.

DUSTING, G.J. and MULLINS, E.M. (1980). Clin. Exp. Pharmacol. Physiol. 7: 545-550.

DUSTING, G.J., MULLINS, E.M. and NOLAN, R.D. (1981). Eur. J. Pharmacol. 70: 129-137.

DUSTING, G.J. (1981). J. Cardiovasc. Pharmacol. 3: 197-206.

EAD, H.W., GREEN, J.H. and NEIL, E. (1952). J. Physiol. 118: 509-519.

EARLEY, L.E. and FRIEDLER, R.M. (1965). J. Clin. Invest. 44: 929-941.

EARLEY, L.E. and FRIEDLER, R.M. (1966). J. Clin. Invest. 45: 542-551.

ERDÖS, E.G. (1977). Fed. Proc. 36: 1760-1770.

FAGARD, R., AMERY, A., LIJNEN, P. and REYBROUCK, T. (1977). Eur. J. Clin. Invest. 7: 473-479.

FEIGEN, L.P., KLAINER, E., CHAPWICK, B.M. and KADOWITZ, P.J. (1976). J. Pharmacol. Exp. Ther. 198: 457-463.

FEJES-TOTH, G., MAHYAR, A. and WALKER, J. (1977). Amer. J. Physiol. 232: F416-F421.

FERREIRA, S.H. (1965). Brit. J. Pharmacol. 24: 163-169.

FERREIRA, S.H. and VANE, J.R. (1967). Nature. 216: 868.

FERREIRA, S.H., BARTLETT, D.C. and GREENE, W. (1970). Biochem. 9: 2583-2593.

FINE, L.G. and TRIZNA, W. (1977). Amer. J. Physiol. 232: F383-F390.

FISCHER, S., SCHERER, B. and WEBER, P.C. (1983). Biochem. Biophys. Acta. 750: 127-133.

FITZGERALD, G.A., HOSSMAN, V., HUMMERICH, W. and KONRADS, A. (1980). Prostaglandins Med. 5: 445-456.

FITZGERALD, G.A., BRASH, A.R., FALARDEAU, P. and OATES, J.A. (1981). J. Clin. Invest. 68: 1272-1276.

FLOWER, R.J. (1974). Pharmacol. Rev. 26: 33-67.

FRAME, M.H., HEDQUIST, R. and ASTRÖM, A. (1974). Life Sci. 15: 239-244.

FRANCISCO, L.L., OSBORNE, J.L. and DI BONA, G.F. (1982). Amer. J. Physiol. 243: F537-F541.

FREEMAN, R.H., DAVIS, J.O., DIETZ, J.R., VILLARREAL, D., SEYMOUR, A.A. and ECHTENKAMP, S.F. (1982). Hypertension. 4: 106-112.

FRESINGER, G.C. (1977). Clin. Res. 25: 41A.

FROLICH, J.C., WILSON, T.W., SWEETMAN, B.J., SMIGEL, M., NIES, A.S., CARR, K., WATSON, J.T. and OATES, J.A. (1975). J. Clin. Invest. 55: 763-770.

FROLICH, J.C., HOLLIFIELD, J.W., DORMAIS, J.C., FROLICH, B.L., SEYBERTH, H., MICHELAKIS, A.M. and OATES, J.A. (1976). Circ. Res. 39: 447-450.

FROLICH, J.C., HOLLIFIELD, J.W., MICHELAKIS, A.M., VESPER, B.S., WILSON, J.P., SHAND, D.G., SEYBERTH, H.J., FROLICH, W.H. and OATES, J.A. (1979). Circ. Res. 44: 781-787.

FULGRAFF, G. and MEIFORTH, A. (1971). Pflügers. Arch. Physiol. 330: 243-249.

GALLER, M., BACKENROTH, R., FOLKERT, V.W. and SCHLONDORFF, D. (1982). J. Pharmacol. Exp. Ther. 220: 23-28.

GANONG, W.F. (1973). Fed. Proc. 32: 1782-2784.

GAVRAS, H., BRUNNER, H.R. and TURIN, G.A. (1978). New Eng. J. Med. 298: 991-995.

GERBER, J.G., BRANCH, R.A., NIES, A.S., GERKENS, J.F., SHAND, D.G., HOLLIFIELD, J. and OATES, J.A. (1978a). Prostaglandins. 15: 81-88.

GERBER, J.G., NIES, A.S., FRIESINGER, G.C., GERKENS, J.F., BRANCH, B.A. and OATES, J.A. (1978b). Prostaglandins. 16: 519-528.

GERBER, J.G., KELLER, R.T. and NIES, A.S. (1979). Circ. Res. 44: 796-799.

* FREELANDER, A.E. and GOODFRIEND, T.L. (1977). Fed. Proc. 36: 481.

* FREELANDER, A.E., KIRSCHBAUM, H., RIS, H., OKEN, D.E. and CANDELORA, P.D., (1980). Clin. Res. 28: 559.

GERBER, J.G., NIES, A.S. and OLSEN, R.D. (1981). J. Physiol. 319: 419-429.

GIMBRONE, M.A. and ALEXANDER, R.W. (1975). Sci. 189: 219-222.

GRANS, K.R. and WONG, P.Y.K. (1981). Fed. Proc. 40: 681.

GRANSTRÖM, E. and SAMUELSSON, B. (1971). J. Biol. Chem. 246: 7470-7473.

GRANSTRÖM, E. (1972). Eur. J. Biochem. 27: 462-464.

GRANTHAM, J. and ORLOFF, I. (1968). J. Clin. Invest. 47: 1154-1161.

GRENIER, F.C. and SMITH, W.L. (1978). Prostaglandins. 16: 759-772.

GRENIER, F.C., ROLLINS, T.E. and SMITH, W.L. (1981). Amer. J. Physiol. 241: F94-F104.

GRODZINSKA, L. and GRYGLEWSKI, R.J. (1980). Pharmacol. Res. Commun. 12: 339-347.

GROSS, F., LAZAR, J. and ORTH, H. (1972). Sci. 175: 656.

GROSS, J.B. and BARTTER, F.C. (1973). Amer. J. Physiol. 225: 218-224.

GRYGLEWSKI, R.J., KORBUT, R. and OCETKIEWICZ, A. (1978). Nature. 273: 765-767.

GULLNER, H.G., NICOLAOU, K.C. and BARTTER, F.C. (1980). Prostaglandins Med. 4: 141-146.

GUYTON, A.C. LANGSTON, J.B. and NAVAR, G. (1964). Circ. Res. 14/15: 187S-196S.

HABER, E. (1976). Circ. 54: 849-861.

HALL, J.E., GUYTON, A.C., TRIPPODO, N.C., LOHMEIER, T.E., McCAA, R.E. and COWLEY, A.W. (1977). Amer. J. Physiol. 232: F538-F544.

HALL, J.E., COLEMAN, T.G., GUYTON, A.C., WILLIAMSON-BALFE, J. and SALGADO, H.C. (1979)a Amer. J. Physiol. 236: F252-F259.

HALL, J.E., GUYTON, A.C., SMITH, M.J. and COLEMAN, T.G. (1979)b Amer. J. Physiol. 237: F424-F432.

HAMBERG, M. (1968). Eur. J. Biochem. 6: 135-137.

HAMBERG, M. and SAMUELSSON, B. (1971). J. Biol. Chem. 246: 6713-6717.

GRANGER, P., DOLHEIM, H. and THURAU, K. (1972). Kidney Int. 1: 78-88.

GREEN, K. (1969). Chem. Phys. Lipids, 3: 254-272.

HARRIS, P.J. and YOUNG, J.A. (1977). Pflüeg. Arch. 367: 295-297.

HASSID, A., KONIECZKOWSKI, M. and DUNN, M.J. (1979). Proc. Nat. Acad. Sci. 76: 1155-1156.

HASSID, A. and DUNN, M.J. (1980). J. Biol. Chem. 255: 2472-2476.

HAYLOR, J. and TOWERS, J. (1982). Brit. J. Pharmacol. 76: 131-137.

HILL, T.W.K. and MONCADA, S. (1979). Prostaglandins. 17: 87-98.

HOCKEL, G.M. and COWLEY, A.W. (1980). In: "Adv. Prostaglandin and Thromboxane Res.". ed., Ramwell, P.W., Samuelsson, B. and Paoletti, P. Vol. 7: 1061-1065.

HOLLENBERG, N.K., WILLIAMS, G.H., BURGER, B., ISHIKAWA, I. and ADAMS, D.F. (1976). J. Clin. Invest. 57: 39-46.

HOLLENBERG, N.K., WILLIAMS, G.H., TAUB, K.J., ISHIKAWA, I., BROWN, C. and ADAMS, D.F. (1977). Kidney Int. 12: 285-293.

HOLLENBERG, N.K., MEGGS, L.G., WILLIAMS, G.H., KATZ, J., GARNIC, J.D. and HARRINGTON, D.P. (1981). Kidney Int. 20: 240-245.

HONG, S.L. (1980). Thromb. Res. 18: 787-795.

HORTON, E. (1979). In: "Chem. Biochem. and Pharmacol. Activity of Prostanoids". ed., Roberts, S. and Scheinmann, F. 1-16.

HOULT, J.R.S. and MOORE, P.K. (1977). Brit. J. Pharmacol. 61: 615-626.

IINO, Y. and IMAI, M. (1978). Pflueg. Arch. 373: 125-132.

IINO, Y. and BRENNER, B.M. (1981). Prostaglandins. 22: 715-721.

JACKSON, B.A., EDWARDS, R.M. and DOUSA, T.P. (1980). J. Lab. Clin. Med. 96: 119-128.

JACKSON, E.K., HERZER, W.A., ZIMMERMAN, J.B., BRANCH, R.A., OATES, J.A. and GERKENS, J.F. (1981). J. Pharmacol. Exp. Ther. 216: 24-28.

JACKSON, E.K., GERKENS, J.F., BRASH, A.R. and BRANCH, R.A. (1982). J. Pharmacol. Exp. Ther. 222: 410-415.

JACKSON, E.K., GOODMAN, R.P., FITZGERALD, G.A., OATES, J.A. and BRANCH, R.A. (1982). J. Pharmacol. Exp. Ther. 221: 183-187.

JANSEN, F.H.A. and NUTERGEN, D.H. (1971). Histochemie. 27: 159-162.

JOHNSON, M.D. and MALVIN, R.L. (1975). Amer. J. Physiol. 229: 1098-1111.

HARVEY, R.B. and BROTHERS, J.A. (1962). Anals N.Y. Acad Sci. 102: 46 - 54.

HEYROUSKY, A. (1956). Clin. Chim. Acta 1: 471.

JOHNSTON,C.I., MILLAR, J.A., McGRATH, B.P. and MATTHEWS, P.G. (1979). Lancet ii: 493-496.

JOHNSTON, C.I., CLAPPISON, B.H., ANDERSON, W.P. and YASIYIMA, M. (1982). Amer. J. Cardiol. 49: 1401-1404.

JOHNSTON, P.A., BERNARD, D.B., PERRIN, N.S., ARBEIT, L., LIBERTHAL, W. and LEVINSKY, N.G. (1981). Circ. Res. 48: 728-733.

JONES, R.L., WATSON, M.L. and UNGAR, A. (1981). Quart. J. Exp. Physiol. 66: 1-15.

KALDOR, A., GACHALYI, B., KALLAU, K., FEKETE, S. and FOLDRARI, A. (1975). Int. J. Clin. Pharmacol. 11: 10-14.

KATHER, H. and SIMON, B. (1979). J. Clin. Invest. 64: 609-612.

KEETON, T.K., PETTINGER, W.A. and CAMPBELL, W.B. (1976). Circ. Res. 38: 531-539.

KEETON, T.K. and CAMPBELL, W.B. (1980). Pharmacol. Rev. 32: 1-81.

KIMBROUGH, H.M., VAUGHAN, D., CAREY, R.M. and AYERS, C.R. (1977). Circ. Res. 40: 174-178.

KIRCHENBAUM, M.A. and STEIN, J.H. (1976). J. Clin. Invest. 57: 517-521.

KOPP, U., AURELL, M., NILSON, I.M. and ABLAD, B. (1980). Pflüeg. Arch. 387: 107-113.

KOPP, U., AURELL, M., SJÖLANDER, M. and ABLAD, B. (1981). Pflüeg. Arch. 391: 1-8.

KREISBERG, J.I. (1982). Kidney Int. 22: 355-359.

LARSSON, C. and ANGGARD, E. (1973). Eur. J. Pharmacol. 21: 30-36.

LARSSON, C. and ANGGARD, E. (1974). Eur. J. Pharmacol. 25: 327-334.

LARSSON, C., WEBER, P.C., ANGGARD, E. (1974). Eur. J. Pharmacol. 28: 391-394.

LARSSON, C. and ANGGARD, E. (1976). J. Pharm. Pharmacol. 28: 326-328.

LEE, J.B., COVINO, B.G. and TAKMAN, B.H. (1965). Circ. Res. 17: 57-77.

LEE, J.B., CROWSHAW, K., TAKMAN, B.H., ATTREP, K.A. and GOUGOUTAS, J.Z. (1967). Biochem. J. 105: 1251-1254.

LEVINSKY, N.G. (1979). Circ. Res. 44: 441-451.

LEWY, J.E. and WINDHANGER, E.E. (1968). Amer. J. Physiol. 214: 943-954.

LIFSCHITZ, M.D., PATAK, R.V., RADEM, S.Z. and STEIN, J.H. (1978). Prostaglandins. 16: 607-619.

LOHMEIER, T.E., COWLEY, A.W., TRIPPODO, N.C., HALL, J.E. and GUYTON, A.C. (1977). Amer. J. Physiol. 233: F388-F395.

LONIGRO, A., ITSKOVITZ, H.D., CROWSHAW, K. and McGIFF, J.C. (1973). Circ. Res. 32: 712-717.

LUM, G.M., AISENBREY, C.A., DUNN, M.J., BERL, T., SCHRIER, R.W. and McDONALD, K.M. (1977). J. Clin. Invest. 59: 8-13.

LUUKAINER, T., VANDEN-NEUVEL, W., HAAHTI, W. and HORNING, E. (1961). Biochem. Biophys. Acta. 52: 599-601.

MALIK, K.U. and McGIFF, J.C. (1975). Circ. Res. 36: 599-602.

MALVIN, R.L. and VANDER, A.J. (1967). Amer. J. Physiol. 213: 1205-1208.

MARSHALL, G.R., VINE, W. and NEEDLEMAN, P. (1970). Proc. Nat. Acad. Sci. 67: 1624-1630.

MATTHEWS, P.G. and JOHNSON, C.I. (1980). Clin. Exp. Pharmacol. Physiol. 7: 62-66.

McCAA, R.E., HALL, J.E. and McCAA, C.S. (1978). Circ. Res. 43: 1S-32S.

McCAA, R.E. (1979). Fed. Proc. 38: 2783.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. and LONIGRO, A.J. (1970)a 26: 121S-126S.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. and LONIGRO, A.J. (1970)b Circ. Res. 27: 121S-130S.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A., LONIGRO, A.J., STRAND, J.C., WILLIAMSON, M.A., LEE, J.B. and NG, K.K.F. (1970). Circ. Res. 27: 765-782.

McGIFF, J.C., TERRAGNO, N.A., MALIK, K.U. and LONIGRO, A.J. (1973). Circ. Res. 31: 36-43.

McGIFF, J.C., SPOKAS, E.G. and WONG, P.Y.K. (1982). Brit. J. Pharmacol. 75: 137-144.

MacGREGOR, G.A. and DAVIES, P.M. (1976). Brit. J. Pharmacol. 3: 483-487.

MacGREGOR, G.A., MARKANDU, N.D., ROULSTON, J.E. and JONES, J.C. (1981). Nature. 291: 329-331.

McGUIRE, J.C. and SUN, F.F. (1978). Arch. Biochem. Biophys. 189: 92-96.

MENDELSOHN, F.A.O. (1979). Clin. Sci. 57: 173-179.

MICHELAKIS, A.M. and McALLISTER, R.G. (1972). J. Clin. Endocrinol. Metab. 34: 386-394.

MIYAMORI, I., BROWN, M.J. and DOLLERY, C.T. (1980). Clin. Exp. Hypertens. 2: 935-945.

MOHAMMED, G., DISCALA, J. and STEIN, R.M. (1974). Amer. J. Physiol. 227: 469-476.

MONCADA, S., GRYGLEWSKI, R., BUNTING, S. and VANE, J.R. (1976). Nature. 263: 663-665.

MONCADA, S., KORBUT, R., BUNTING, S. and VANE, J.R. (1978). Nature. 273: 767-768.

MOORE, T.J., CRANTZ, F.R., HOLLENBERG, N.K., KOTESKY, R.J., LEBOFF, M.S., SWARTZ, S.L., LEVINE, L., PODOLSKY, S., DLUHY, R.G. and WILLIAMS, G.H. (1981). Hypertension. 3: 168-173.

MOORE, P.K. and HOULT, J.R.S. (1978). Biochem. Biophys. Acta. 528: 276-279.

MORRISON, A.R., NISHIKAWA, K. and NEEDLEMAN, P. (1977). Nature. 267. 259-60.

MORTON, J.J., TREE, M. and CASALS-STENZEL, J. (1982). Amer. J. Cardiol. 21: 1395-1340.

MUIRHEAD, E.E., PREWITT, R.L., BROOKS, B. and BROSIUS, W.L. (1978). Circ. Res. 43: 53-59.

MUIRHEAD, E.E., BROOKS, B. and BROSIUS, W.L. (1980). Arch. Pathol. Med. 104: 631-634.

MULLANE, K.M. and MONCADA, S. (1980). Eur. J. Pharmacol. 66: 355-365.

MULLANE, K.M. and MONCADA, S. (1980). Prostaglandins. 20: 25-49.

MURTHY, V.S., WALDRON, T.L. and GOLDBERG, M.E. (1978). Circ. Res. 43: 1S-40S.

NAKANO, J. and MORSY, N.H. (1971). Clin. Res. 19: 142-145.

NASJLETTI, A., COLINA-CHURITO , J. and McGIFF, J.C. (1975). Circ. Res. 37: 59-65.

NASJLETTI, A. and MALIK, K.U. (1982). Fed. Proc. 41: 2394-2399.

MONCADA, S. and VANE, J.R., (1978). Pharmacol. Rev. 30: 293-331.

NAVAR, L.G. and LANGFORD, H.G. (1974). In: "Angiotensin". ed., Page, I. and Bumpus, F.M. 455-474.

NEEDLEMAN, P., KAUFFMAN, A.H., DOUGLAS , J.R., JOHNSON, E.M. and MARSHALL, G.R. (1973). Amer. J. Physiol. 224: 1415-1419.

NEEDLEMAN, P., MARSHALL, G.R. and SOBEL, B.E. (1975). Circ. Res. 37: 802-808.

NEEDLEMAN, P., BRONSON, S.D., WYCHE, A. and SIVAKOFF, M. (1978). J. Clin. Invest. 61: 839-849.

NEGUS, P., TANNEN, R.L. and DUNN, M.J. (1976). Prostaglandins. 12: 175-180.

NIARCHOS, A.P., PICKERING, T.G., CASE, D.B., SULLIVAN, P. and LARAGH, J.H. (1979). Circ. Res. 45: 829-831.

NOLAN, R.D., DUSTING, G.J. and MARTIN, T.J. (1981). Biochem. Pharmacol. 30: 2121-2125.

NORBIATO, G.A., BAVILACQUA, M., RAGGI, V., MICOSSI, P. MOROUI, C. and FASOLI, A. (1978). Acta. Endocrinol. 87: 577-588.

NORTH, R.H., TAN, S.Y. and MULROW, P.J. (1977). J. Endocrinol. 45: 10-15.

OKAHARA, T., IMANISHI, M. and YAMAMOTO, K. (1983). Prostaglandins. 25: 373-385.

OLIVER, J.A., SCIACCA, R.R. and CANNON, P.J. (1979). Amer. J. Physiol. 236: H427-H433.

OLIVER, J.A., PINTO, J., SCIACCA, R.R. and CANNON, P.J. (1980). J. Clin. Invest. 66: 748-756.

OLIVER, J.A., SCIACCA, R.R. and CANNON, P.J. (1983). Hypertension. 5: 166-171.

OLIW, E., LUNDEN, I., SJOQUIST, B. and ANGGARD, E. (1979). Acta. Physiol. Scand. 105: 359-366.

OLSEN, U.B. and ARRIGONI-MARTELLI, E. (1979). Eur. J. Pharmacol. 54: 229-234.

OLSEN, R.D., NIES, A.S. and GERBER, J.G. (1981). J. Pharmacol. Exp. Ther. 219: 321-325.

ONDETTI, M.A., WILLIAMS, N.J., SABO, E.F., PLUSAC, J., WEAVER, E.R. and KOCY, O. (1971). Biochem. 10: 4033-4039.

ONDETTI, M.A., RUBIN, B. and CUSHMAN, D.W. (1977). Sci. 196: 441-444.

OPGENORTH, T.J., WAPPEL, M. and ZEHR, J.E. (1980). Fed. Proc. 39: 497-451.

ORSTAVIK, T.B., NUSTAD, K., BRANDTZAEG, P. and PIERCE, J.V. (1976). J. Histochem. Cytochem. 24: 1037-1039.

OSBORN, J.C., NOORDEINER, B., HOOK, J.B. and BAILIE, M.D. (1978). Proc. Soc. Exp. Biol. Med. 159: 249-251.

PACE-ASCIAK, C.R. (1975). J. Biol. Chem. 250: 2789-2795.

PACE-ASCIAK, C.R., CARRARA, M.C., LEVINE, L. and NICOLAOU, K.C. (1980). Prostaglandins. 20: 1053-1060.

PALS, D.T., MASUCCI, F.D., SIDPOS, F. and DENNING, G.S. (1971). Circ. Res. 29: 664-672.

PATRONO, C., CIABATTONI, G., , PUGLIESE, F., MASERI, A. and CHIERCHIA, S. (1979). Clin. Res. 27: 426A.

PATRONO, C., PUGLIESE, F., CIABATTONI, G., MASERI, A., CHIERCHIA, S., PESKAR, B.A., CINOTTI, G.A., SIMONETTI, B.M. and PIERUCCI, A. (1982). J. Clin. Invest. 69: 231-239.

PAULSEN, K., BURTON, J. and HABER, E. (1973). Biochem. 12: 3877-3880.

PETRULIS, A.S., AIKAWA, M. and DUNN, M.J. (1981). Kidney Int. 20: 469-474.

PIKE, J.E., KUPIECKI, F.F. and WEEKS, J.R. (1967). In: "Prostaglandins". ed., Bergström, S. and Samuelsson, B. 161-682.

PIPER, P.J. and VANE, J.R. (1969). Nature. 239: 29-30.

QUILLEY, C.P., WONG, P.Y.K. and McGIFF, J.C. (1979). Eur. J. Pharmacol. 57: 273-276.

RATHAUS, M., PODJARNY, E., WEISS, E., RAVID, M., BAUMINGER, S. and BERNHEIM, J. (1981). Clin. Sci. 60: 405-410.

RAY, C. and MORGAN, T. (1981). Pflüeg. Arch. 392: 51-56.

RENNICK, B.R. (1977). Amer. J. Physiol. 233: F133-F137.

ROMERO, J.C., STANELONI, R.J., DUFAN, M.L., DOHMEN, R., BINIA, A., KLIMAN, B. and FASCIOLO, J.C. (1968). Metab. 17: 10-19.

ROMERO, J.C., HOOBLER, S.W., KOZAK, T.J. and WARZYNSKI, R.J. (1973). Amer. J. Physiol. 225: 810-814.

ROMERO, J.C., DUNLOP, C.L. and STRONG, C.G. (1976). J. Clin. Invest. 58: 282-288.

ROSENBLATT, S.G., PATAK, R.V. and LIFSCHITZ, M.D. (1978). Amer. J. Physiol. 235: F473-F479.

ROSENKRANZ, B., FISCHER, C., WELMER, K.E. and FROLICH, J.C. (1980)a J. Biol. Chem. 255: 10194-10198.

ROSENKRANZ, B., FISCHER, C., REIMANN, I., WEIMER, K.E., BECK, G. and FROLICH, J.C. (1980)b Biochem. Biophys. Acta. 619: 207-213.

ROSENKRANZ, B., KITAJIMA, W. and FROLICH, J.C. (1981)a Kidney Int. 19: 755-759.

ROSENKRANZ, B., FISCHER, C. and FROLICH, J.C. (1981)b Clin. Pharmacol. Ther. 29: 420-424.

RUMPF, K.W., FRENZEL, S., LOWITZ, H.D. and SCHELER, F. (1975). Prostaglandins. 10: 641-648.

SALVETTI, A., ARZILLI, F., PEDINELLI, R. and BEGGI, P. (1980). Drugs Exp. Clin. Res. 6: 541-551.

SATOH, H. and SATOH, S. (1980). Biochem. Biophys. Res. Commun. 94: 1266-1272.

SCHARSCHMIDT, L.A. and DUNN, M.J. (1983). J. Clin. Invest. 71: 1756-1776.

SCHERER, B., SCHNERMANN, J., SOFRONIEV, M. and WEBER, P.C. (1978). Prostaglandins. 15: 255-265.

SCHLENK, H. and GELLERMAN, J. (1960). Anal. Chem. 32: 1412-1414.

SCHLONDORFF, D., ROCZNIAK, S., SATRIANO, J.A. and FOLKERT, V.W. (1980). Amer. J. Physiol. 239: F486-F495.

SCHLONDORFF, D., ZANGER, R., SATRIANO, J.A., FOLKERT, V.W. and EVELOFF, J. (1982). J. Pharmacol. Exp. Ther. 223: 120-124.

SCHMIDT, H.E. (1962). Circ. Res. 11: 185-193.

SCHNERMANN, J., SCHUBERT, G., HERMLE, M., HERBST, R., STOWE, N.T., YARIMIZU, S. and WEBER, P.C. (1979). Pflüg. Arch. 379: 269-286.

SCHNERMANN, J. and WEBER, P.C. (1982). Prostaglandins. 24: 351-356.

SCHRIER, R.W. and De WARDENER, H.E. (1971). New Eng. Med. J. 285: 1231-1243.

SCHWERTSCHLAG, U., STAHL, T. and HACKENTHAL, E. (1982). Prostaglandins. 23: 129-138.

SELDIN, D.W., EKNOYAN, G., SUKI, W.N. and RECTOR, F.C. (1966). Annals. N.Y. Acad. Sci. 139: 329-343.

SEYMOUR, A.A. and ZEHR, J.E. (1979). Circ. Res. 45: 13-25.

SEYMOUR, A.A. and DAVIS, J.O. ECHENKAMP, S.F., DIETZ, J.R. and FREEMAN, R.H. (1981) Amer. J. Physiol. 240: F 515 - F 521.

SHANNON, J.A. and SMITH, H.W. (1935). J. Clin. Invest. 14: 393-401.

SHEBUSKI, R.J. and AIKEN, S.W. (1980). J. Cardiovasc. Pharmacol. 2: 667-677.

SIESS, W. and DRAY, F. (1982). J. Lab. Clin. Med. 99: 388-398.

SILBERBAUER, K. and SINZINGER, H. (1978). Thromb. Res. 13: 1111-1118.

SILBERBAUER, K., SINZINGER, H. and WINTER, M. (1979). Exp. Mol. Pathol. 30: 242-254.

SILBERBAUER, K., STANEK, B. and TEMPL, H. (1982). Brit. J. Clin. Pharmacol. 14: 87S-93S.

SIROIS, P. and GAGNON, D.J. (1974). Eur. J. Pharmacol. 28: 18-24.

SPECKHART, P., ZIA, P., ZISPER, R. and HORTON, R. (1977). Clin. Endocrinol. Metab. 44: 832-836.

SMITH, W.L. and WILKIN, G.P. (1977). Prostaglandins. 13: 873-892.

SMITH, W.L. and BELL, T.G. (1979). Prostaglandins. 18: 269-273.

SMITH, W.L. and BELL, T.G. (1978). Amer. J. Physiol. 235: F451-F457

SMITH, H.W., FINKELSTEIN, N., ALIMINOSA, L., CRANFORD, B. and GRABER, M. (1945). J. Clin. Invest. 24: 388-404.

SRAER, J., SRAER, J.D., CHANSEL, D., RUSIO-MARIE, F., KOUZNETZORA, B. and ARDOULLOU, R. (1979). Mol. Cell. Endocrinol. 16: 29-37.

SRAER, J., SIESS, W., MOULONQUET-DOLERIS, L., OUDINET, J.P., DRAY, F. and ARDOULLOU, R. (1982). Biochem. Biophys. Acta. 710: 45-52.

STAHL, R.A.K., AHMAD, A., ATTALLAH, D.L., BLOCH, D.L. and LEE, J.B. (1979). Amer. J. Physiol. 237: F344-F349.

STEIN, J.H., CONGBALAY, R.C., KARSH, D.L., OSGOOD, R.W. and FERRIS, T.F. (1972). J. Clin. Invest. 51: 1709-1721.

STEIN, J.H., OSGOOD, R.W. and KUNAN , R.T. (1976). J. Clin. Invest. 58: 767-773.

STOKES, J.B. and KOKKO, J. (1977). J. Clin. Invest. 59: 1099-1104.

STOKES, J.B. (1979). J. Clin. Invest. 64: 495-502.

STONE, K.J. and HART, M. (1975). Prostaglandins. 10: 273-276.

STRANHOY, J.W., OTT, C.E., SCHNEIDER, E.G., WILLIS, L.R., BECK, N.P., DAVIS, B.B. and KNOX, F.G. (1974). Amer. J. Physiol. 226: 1015-1021.

STRONG, C.G., BOUCHER, R., NOWACZYNSKI, W. and GENEST, J. (1966). Mayo Clinic Proc. 41: 433-452.

SUN, F.F. and TAYLOR, B.M. (1978). Biochem. 17: 4096-4103.

SUN, F.F., TAYLOR, B.M., SUTTER, D.M. and WEEKS, J.R. (1979). Prostaglandins. 17: 753-759.

SUN, F.F. and TAYLOR, B.M. (1981). Prostaglandins. 21: 307-314.

SUN, F.F., TAYLOR, B.M., McGUIRE, J.C. and WONG, P.Y.K. (1981). Kidney Int. 19: 760-770.

SUZUKI, S., FRANCO-SAENZ, R., TAN, S.Y. and MULROW, P.J. (1981)a Proc. Soc. Exp. Biol. Med. 166: 484-488.

SUZUKI, S., FRANCO-SAENZ, R., TAN, S.Y. and MULROW, P.J. (1981)b Amer. J. Physiol. 240: E286-E289.

SWARTZ, S.L., WILLIAMS, G.H., HOLLENBERG, N.K., LEVINE, L., DLUHY, R.G. and MOORE, T.J. (1980). J. Clin. Invest. 65: 1257-1264.

SWIESS, J., RADOMSKI, M. and GRYGLEWSKI, R.J. (1979). Pharmacol. Res. Commun. 11: 649-655.

SZCZEKLIK, J., SZCZEKLIK, A. and NIZANKOWSKI, R. (1980). In: "Adv. Prostaglandin and Thromboxane Res.". ed., Ramwell, P. and Samuelsson, B. Vol. 7: 769-775.

TANNENBAUM, J., SPLAWINSKI, J.A., OATES, J.A. and NIES, A.S. (1975). Circ. Res. 36: 197-203.

TAYLOR, B.M., SHEBUSKI, R.J. and SUN, F.F. (1983). J. Pharmacol. Exp. Ther. 224: 692-698.

TERRAGNO, N.A., TERRAGNO, A. and McGIFF, J.C. (1977). Circ. Res. 40: 590-595.

TERRAGNO, N.A., McGIFF, J.C. and TERRAGNO, A. (1978). Clin. Res. 26: 545A.

THURAU, K. (1963). 2nd Int. Cong. Nephrol. Exerpta Medica. 51-61.

THURSTON, H. and LARAGH, J.H. (1975). Circ. Res. 36: 113-117.

TOBIA, A.J. and GIARDINO, E.C. (1981). Proc. Soc. Exp. Biol. Pharmacol. Corp. 167: 242-247.

TOBIAN, L. and O'DONNEL, M. (1976). Fed. Proc. 35: 2388-2392.

TREE, M. and MORTON, J.J. (1980). Clin. Sci. 59: 451-456.

TRIPPODO, N.C., HALL, J.E., LOHMEIER, T.E. and GUYTON, A.C. (1977). Clin. Sci. Mol. Med. 52: 454-458.

TURKER, R.K., HALL, M.M., YAMAMOTO, M., SWEET, C.S. and BUMPUS, F.M. (1972). Sci. 177: 1203-1205.

VANDER, A.J. and GEELHOED, G.W. (1965). Proc. Soc. Exp. Biol. Med. 120: 399-403.

VANDER, A.J. (1968). Amer. J. Physiol. 214: 218-222.

VANDOGEN, R. and PEART, W.S. (1974). Clin. Sci. Mol. Med. 47: 471-479.

VANDOGEN, R., TUNNEY, A. and MARTINEZ, P. (1981). Clin. Sci. 60: 387-392.

VANDOGEN, R., TUNNEY, A., MAHONEY, D. and BARDEN, A. (1981). Prostaglandins. 21: 1007-1014.

VANDOGEN, R., TUNNEY, A., BARDEN, A. and MAHONEY, D. (1982). Hypertension. 4: 642-645.

VIO, C.P., GUIVERNAU, M., TERRAGNO, A. and TERRAGNO, N.A. (1981). Fed. Proc. 40: 702-706.

WEBER, P.C., HOLZGREVE, H., STEPHAN, R. and HERBST, R. (1975). Eur. J. Pharmacol. 34: 299-304.

WEBER, P.C., LARSSON, C., ANGGARD, E., HAMBERG, M., COREY, E.J., NICOLAOU, K.C. and SAMUELSSON, B. (1976). Circ. Res. 39: 868-872.

WEBER, P.C., LARSSON, C. and SCHERER, B. (1977). Nature. 266: 65.

WEEKS, J.R. and COMPTON, L.D. (1979). Prostaglandins. 17: 501-513.

WERNING, C., VETTER, W., WEIDMANN, P., SCHWEIKERT, H.U., STIEL, D. and SIEGENTHALER, W. (1971). Amer. J. Physiol. 220: 852-856.

WHORTON, A.R., MISONO, K., HOLLIFIELD, J., FROLICH, J.C., INAGANII, T. and OATES, J.A. (1977). Prostaglandins. 14: 1095-1099.

WHORTON, A.R., SMIGEL, M., OATES, J.A. and FROLICH, J.C. (1978). Biochem. Biophys. Acta. 529: 176-180.

WHORTON, A.R., LAZAR, J.D., SMIGEL, M.D. and OATES, J.A. (1981). Prostaglandins. 22: 455-468.

WHORTON, A.R., YOUNG, S.L., DATA, J.L., BARCHOWSKY, A. and KENT, R.S. (1982). Biochem. Biophys. Acta. 712: 79-87.

WILLIAMS, W.M., FROLICH, J.C., NIES, A.S. and OATES, J.A. (1977). Kidney Int. 11: 256-260.

WILSON, T.W., LOADHOLT, C.B., PRIVITERA, P.J. and HALUSHKA, P.V. (1982). Hypertension. 4: 634-641.

WITZGALL, H., SCHERER, B. and WEBER, P.C. (1982). Clin. Sci. 63: 265S-267S.

WONG, P.Y., SUN, F.F. and McGIFF, J.C. (1978). J. Biol. Chem. 253: 5555-5557.

WONG, P.Y.K., McGIFF, J.C., CAGEN, L., MALIK, K.U. and SUN, F.F. (1979). J. Biol. Chem. 254: 12-14.

WONG, P.Y.K., LEE, W.H., REISS, R.F. and McGIFF, J.C. (1980). Fed. Proc. 39: 392-395.

WONG, P.Y.K., MALIK, K.U., DESIDERIUS, D.M., McGIFF, J.C. and SUN, F.F. (1980). Biochem. Biophys. Res. Commun. 93: 486-494.

WONG, P.C. and ZIMMERMAN, B.G. (1980)a Life Sci. 27: 1291-1297.

WONG, P.C. and ZIMMERMAN, B.G. (1980)b Exp. Ther. 215: 104-108.

WONG, P.C., ZIMMERMAN, B.G., KRAFT, E., KOUNENUS, G. and FRIEDMAN, P. (1981). J. Pharmacol. Exp. Ther. 219: 646-650.

YOSHINAGA, K., ABE, K., MIWA, I., FUNUJAMA, T. and SUZUKI, C. (1964). Experientia. 20: 396-399.

YUN, J., KELLY, G., BARTTER, F.C. and SMITH, H. (1977). Circ. Res. 40: 459-464.

YUN, J.C., KELLY, G.D., BARTTER, F.C. and TATE, J.E. (1978). Life Sci. 22: 1545-1554.

ZAMBRASKI, E.J. and DUNN, M.J. (1979). Amer. J. Physiol. 236: F552-F558.

ZENSER, T.U., HERMAN, C.A., GORMAN, R.R. and DAVIS, B.B. (1977). Biochem. Biophys. Res. Commun. 79: 357-363.

ZIMMERMAN, B.G. (1978). Prostaglandins. 15: 1027-1034.

ZIMMERMAN, B.G., MOMSEN, C. and KRAFT, E. (1980)a Proc. Soc. Exp. Biol. Med. 164: 459-465.

ZIMMERMAN, B.G., MOMSEN, C. and KRAFT, E. (1980)b In: "Adv. Prostaglandin and Thromboxane Res.". ed., Ramwell, P.W., Samuelsson, B. and Paoletti, R. Vol. 7: 1153-1157.

ZUSMAN, R.M., SPECTOR, D., CADWELL, B.V., SPEROFF, L., SCHNEIDER, G. and MULROW, P.J. (1973). J. Clin. Invest. 52: 1093-1098.

ZUSMAN, R.M. and KEISER, H.R. (1977). J. Biol. Chem. 252: 2069-2071.

Footnote.

The action of the 5mg/kg dose of captopril to inhibit angiotensin 11 formation throughout the period of the study was previously tested in an anaesthetised dog preparation. This was achieved by demonstrating that the pressor response to angiotensin 1 had been abolished.